Polygenic risk score reveals mitochondrial transporter as new target in schizophrenia astrocytes

Hoekstra, Stephanie Danielle

2022

document version
Publisher's PDF, also known as Version of record

Polygenic risk score reveals mitochondrial transporter as new target in schizophrenia astrocytes

Optimizing iPSC-research for genetically complex diseases

Stephanie Beekhuis-Hoekstra

ISBN 978-94-6469-107-8

Cover design: Stephanie Hoekstra
Layout: Bregje Jaspers | ProefschriftOntwerp.nl
Printed by: PorefschriftMaken | www.proefschriftmaken.nl

VRIJE UNIVERSITEIT

Polygenic risk score reveals mitochondrial transporter as new target in schizophrenia astrocytes

Optimizing iPSC-research for genetically complex diseases

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. J.J.G. Geurts,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Bètawetenschappen
op vrijdag 25 november 2022 om 9.45 uur
in een bijeenkomst van de universiteit,
De Boelelaan 1105

door

Stephanie Danielle Hoekstra

geboren te Rio de Janeiro, Brazilië

promotor:	prof.dr. D. Posthuma
copromotor:	dr. V.M. Heine
promotiecommissie:	prof.dr. M.P. van den Heuvel prof.dr. H.E. de Vries prof.dr. E.M. Hol dr. R. Min prof.dr. S. Djurovic

En toen het licht kwam in de ochtend
En de zon die viel door het raam
Lagen jullie in mijn armen
Mama is nu mijn naam

Een lach, een schater,
Stralende oogjes en een kus
Dat is mijn motivatie dus

CONTENTS

Chapter 1:	General introduction	9
Chapter 2:	Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity	45
Chapter 3:	Generation of Isogenic Controls for In Vitro Disease Modelling of X-Chromosomal Disorders	75
Chapter 4:	Systematic assessment of variability in the proteome of iPSC derivatives	107
Chapter 5:	iPSC-derived astrocytes with high polygenic risk reveal novel candidate for convergent effects in schizophrenia	147
Chapter 6:	Myelin forming organoids for the investigation of white matter abnormalities in schizophrenia	183
Chapter 7:	General discussion	217
Appendix:	Dutch Summary	253
	Publications	257
	Acknowledgements	259

Chapter 1A

General Introduction

The term 'schizophrenia' was first used by the psychiatrist Eugene Bleuler, 110 years ago, in an attempt to describe the segregation of cognition, perception and personality. Since that period, many researchers have devoted their efforts into resolving the cause of this disease. This emphasizes the importance and the motivation to understand the underlying biological mechanisms of schizophrenia. Unfortunately, despite the investment of time and effort from over a century of scientific research, these causal mechanisms of schizophrenia are still unknown. To put it into the words of a lecturer at the first Summer School I participated in during my PhD trajectory:

"Decades of scientific history have been written by people NOT solving schizophrenia."

With these encouraging words I dedicated years of my life to create an *in vitro* model in an attempt to understand the role of astrocytes in SCZ pathology. This thesis will describe this journey towards our *in vitro* model and will end with our reports on schizophrenia pathology. However, before describing the research performed as part of my dissertation, I will discuss some valuable findings and prevailing hypotheses that arose during these decades which my lecturer summarized as 'not solving schizophrenia'.

1.1 - FROM CLINICAL PRESENTATION TO THE CAUSALITY OF SCHIZOPHRENIA

Historical description of SCZ

Until the 19th century, it was believed that mental disorders were a punishment by (the) God(s) for sinful behavior or disobedience (Collin et al. 2016). Wilhelm Griesinger argued that such disorders were brain related, and his apprentice Theodor Meynet was the first researcher to suggest that brain dysconnectivity is fundamental in neuropsychiatric diseases. More specifically, he proposed that disorganization of the association fibers and impaired cooperation between frontal areas and other areas of the brain could be the underlying cause of amentia (sudden onset confusion with psychotic symptoms)(Collin et al. 2016). His pupil, Carl Wernicke, further hypothesized that defects or interruptions in the connections between neurons could underlie psychosis. Emil Kraepelin supported this, and was the first to include age of onset, family history and premorbid personality in the diagnosis of dementia praecox. He was the first to find evidence for a hereditary factor in the disorder and that environmental factors could increase the prevalence of psychosis (Collin et al. 2016). Bleuler, who used the term schizophrenia, believed that the underlying mechanisms were not neuronal or cellular but rather disturbances in conduction (Collin et al. 2016). In the first half of the 20th century, psychiatry took a turn towards psychodynamic psychiatry and biologically based psychiatric findings were dismissed as brain mythology due to lack of evidence for brain abnormalities in

psychiatric disorders. During the second half of the 20th century, evidence for the hereditary basis of psychiatric disorders and the antipsychotic treatments led to increased popularity of neurobiology. Eventually, the dysconnectivity theory also reemerged in the field of SCZ, although only in the late 80s (discussed in the section hypotheses in schizophrenia) (Collin et al. 2016). In summary, early studies created the base for the prevailing hypothesis that SCZ arises from altered conductivity of signals between neurons which have now been translated early insights into disturbances in functional connectivity by Collins, Turk and van de Heuvel (2016)

Modern definitions of SCZ and prognosis

Schizophrenia is currently considered to be a neurodevelopmental psychiatric syndrome that affects roughly 1% of the population worldwide (Fleischhacker et al. 2014). Symptomatology includes psychotic symptoms (also known as positive symptoms, e.g. hallucinations and delusions), negative symptoms (such as diminished expressiveness and decreased motivation) and cognitive symptoms (deficits in working memory and impaired executive functions; (Marder and Cannon 2019). Patients usually seek help when positive results become apparent, although negative and cognitive symptoms often precede positive symptoms but generally attract less clinical attention (McCutcheon et al. 2020). Before the diagnosis is made, usually at adolescence, young children can show social awkwardness, physical clumsiness and low intelligence quotients (Marder and Cannon 2019). Closer to clinical diagnosis (usually years or months before), patients often show subtle changes in behavior, such as social withdrawal, decline of professional performance and development of perceptual anomalies. SCZ onset is often claimed to start during adolescence, however, since negative and cognitive symptoms are clearly present before the manifestation of psychotic symptoms, one could argue that the onset of this disease occurs much earlier in development (McCutcheon et al. 2020).

It is hypothesized that schizophrenia arises through a combination of genetic vulnerability and environmental factors, including early-life stress and obstetrical complications (Fromer et al. 2016). The diagnosis of schizophrenia is currently given on the basis of the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV or DMS-V and patients mainly receive antipsychotic medication, giving varying effects. The response in patients, if any, is usually a decrease in positive symptoms without melioration of the negative symptoms, although the latter can even deteriorate (Miyamoto et al. 2012). Needless to say, this has a devastating effect on the lives of the patients and their families. Besides the detrimental effects for the individuals involved, there is also a huge societal burden that comes with this disorder. In Europe, less than 20% of individuals suffering from SCZ are employed (Marwaha et al. 2007). Only 14% of patients recover within the first five years following a psychotic episode, and another 16% after these five years (Harrison et al. 2001; Robinson et al. 2004). The burden

SCZ places at individual level and at the level of society, clearly shows the need for better, more effective treatment.

Environmental risk factors

As mentioned, Emil Kraepelin was the first to link psychosis to environmental factors, which included difficult labor, previous miscarriages, stillborn children and premature births. Research has shown that many environmental factors can increase the prevalence of SCZ, especially during *in utero* development (Kraepelin 1990; Adityanjee et al. 1999). Such factors include maternal infections, maternal starvation, obstetric complications such as preterm birth and preeclampsia. Moreover, Ursini et al. (2018) showed that the prediction of the polygenic risk scores (discussed below) for SCZ was increased 5-fold when considering obstetric risk factors. This neatly shows the interactions between genetic and environmental risk. Exposure to influenza virus, toxoplasmosis and herpes simplex virus type 2 during pregnancy have been associated with increased risk of psychotic disorders (Buka et al. 2001; Brown et al. 2002; Stilo and Murray 2019), but these results remain debated. Fetal malnutrition, prematurity and maternal infections during pregnancy have also been linked to the development of psychotic disorders in later life. Additional paternal factors are increased paternal age while maternal age results in inconsistent findings (McGrath et al. 2014). These findings show that factors as early as prenatal development may contribute to SCZ risk.

Events that take place after prenatal period have also been shown to have an impact on disease risk. Both traumatic events and social adversities during childhood and adulthood have been reported to increase SCZ risk (Marder and Cannon 2019; McCutcheon et al. 2020). Childhood trauma is robustly correlated to positive symptoms in adulthood. Furthermore, life events closer to clinical manifestation have also been investigated and linked life events between 3 months to 3,6 years prior to psychosis onset. Further increase in risk are: loneliness, low social-economic status, migration (especially for refugees) living in an urban environment and substance use (Stilo and Murray 2019). These associations show a clear role for environmental factors in SCZ risk and are summarized in Figure 1. The current hypothesis is that there is an interplay between genes and environment, where genetic variants can make one more vulnerable for environmental triggers. In some cases, the increased risk for SCZ can be inherited over two generations (refugees), showing a potential role for epigenetic modulations. However, correlational evidence does not imply causality in observatory studies, meaning that experimental studies are needed to verify any causal link. Obviously, this type of research is unethical in humans, and limited to SCZ-like symptoms in animal models, complicating the verification of these factors in disease contribution. Nevertheless, when modeling SCZ, environmental factors should be considered.

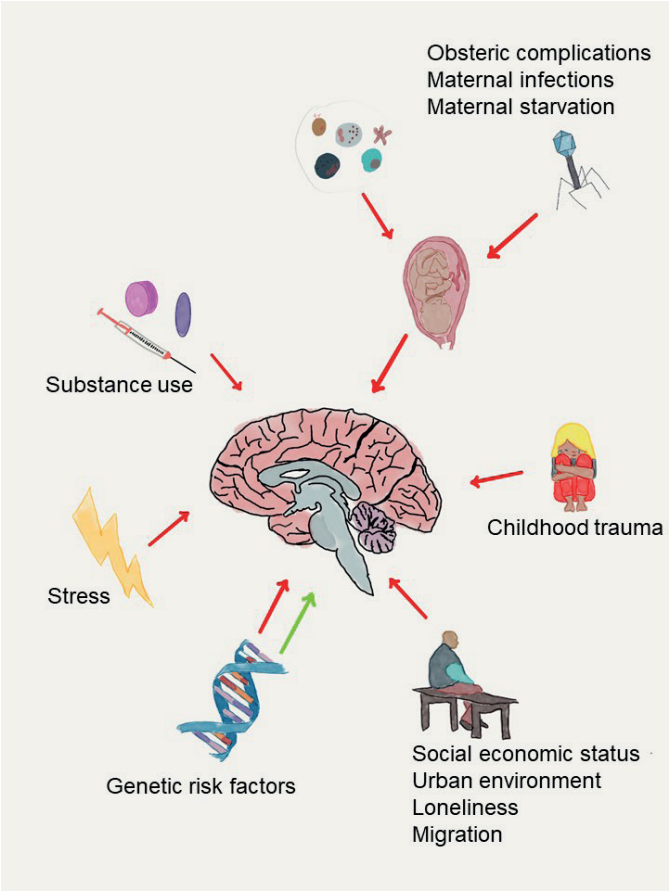


Figure 1: Illustration of factors that are associated with increased risk for developing SCZ. Of note: these are correlational association that do not direct imply causality.

Heterogeneity

As illustrated by its complicated history, schizophrenia presents with a complex set of symptoms that can vary per individual causing high heterogeneity within this group of patients (Miller 2010). Although schizophrenia is mainly seen as one disorder with high heterogeneity, studies have also shown by hierarchical clustering of patients based on elaborate clinical and neurophysiological data that there might be sub classifications within schizophrenia (Wessman et al. 2009). This would be in line with reports from the founding fathers of schizophrenia Kraepelin and Bleuler, who both subcategorized it. The distinction of different types of SCZ may facilitate research as it might reduce the heterogeneity between patients. On the other hand, classification into types might make diagnosis more difficult, as

patients could be diagnosed with several subtypes. The latter is one of the reasons why the new DSM-V is designed as a scale rather than subtypes. This heterogeneity is also seen at genetic level, as different patients carry different SCZ associated variants (this is discussed below and in Chapter 2). While heterogeneity between patients has been appreciated on different levels, the lack of ways to make clear distinctions between different types of SCZ forms an important hurdle for research progress. Therefore, we need better understanding of SCZ and its potential subtypes, in order to facilitate scientific research and treatment strategy.

1.2 - NEXT GENERATION DNA ANALYSIS GAVE NEW INSIGHT IN SCZ PATHOLOGY

Heritability of SCZ

In 1916 Rudin et al. showed that the rate of schizophrenia was higher among family members of patients than among the general population (Kendler and Zerbin-Rüdin 1996). In addition, Gottesman (Gottesman 1991) showed that the incidence of schizophrenia is higher in individuals with an affected first degree relative compared to an affected second degree relative. Since the 1920s researchers compared monozygotic to dizygotic twins to control for a shared environment while estimating the contribution of genetic factors. Studies revealed that monozygotic twins indeed had higher concordance for SCZ than dizygotic twins (Cardno and Gottesman 2000; Henriksen et al. 2017). Further confirmation for a genetic cause came from adoption studies (Heston 1966; Wender et al. 1974). A meta-analysis of twin studies revealed a heritability of 81% and the influence of shared environment was estimated at 11% (Sullivan et al. 2003), but there are studies reporting lower heritability (Lichtenstein et al. 2009). Despite the uncertainty of the exact heritability of SCZ, it is clear that there is a strong genetic component in this disorder.

Genetic analysis actually involving the DNA was made possible after The Human Genome Project, which ran between 1990 and 2003 (Henriksen et al. 2017). The genetic studies started with linkage analysis to identify haplotypes that were prevalent in affected individuals compared to healthy family members or twin pairs. This type of analysis only revealed relatively large loci without implying specific alleles. Numerous studies were able to associate specific loci with schizophrenia spectrum disorders, although all presented different loci which were often not replicated by others due to underpowered studies (Risch and Merikangas 1996). Researchers soon concluded that SCZ is likely polygenic and the genetic factors might have effect sizes that were too small to be found using the sample sizes available at the time (Henriksen et al. 2017). Candidate genes were selected using the information derived from linkage studies (position) and/or functionality (usually genes related to dopamine and serotonin neurotransmission were selected). By now, over 1000 genes have

been tested, generating a couple of known copy number variants (CNVs) such as disrupted in schizophrenia 1 (DISC1) and catehol-O-methyltransferase (COMT) (Henriksen et al. 2017). However, results are still not conclusive as the causality of the correlated genes remains debated. One important drawback of the candidate approach is that it is hypothesis-driven and relies on current knowledge. Since the underlying pathways in SCZ remain unclear, the posed hypotheses on convergent disease mechanisms might also be inaccurate, making it hard to select genes with high confidence. Therefore, the selection of a candidate gene must be done with caution, as inaccurate hypotheses can lead research into the wrong direction. Taken together, after decades of research the heritability of SCZ was shown, although exact loci have not been identified.

GWAS

In 2007, a hypothesis-free approach arose, called Genome Wide Association Studies (GWAS). This approach focuses on single nucleotide polymorphisms (SNPs) and relies on linkage disequilibrium (the association between alleles between at least two loci in the general population). In a GWAS, the enrichment of genetic variants is tested against a phenotype, reflecting a genetic association between that variant and the phenotype tested. GWAS studies have been able to find genetic variants associated with schizophrenia with each only accounting for a small increase in risk. Although the associations have not undergone verification by the candidate genes approach (Henriksen et al. 2017), the newly found variants have been replicated by other GWAS studies and meta-analysis, indicating that they are reliable associations. An important achievement was the identification of 128 schizophrenia associated variants, located in 108 different loci in 2014 (Consortium 2014). Yet, the majority of the genetics in schizophrenia still seems to be 'missing', as the GWAS studies performed so far only explain around 6% of the heritability while an estimate of 81% is assumed. This means that many of the genetic variants leading to schizophrenia still need to be found. When assuming this additive model, the effects of many genetic variants are simply cumulated and the missing heritability is mainly explained by a lack of power or unobserved SNPs (the latter could be the case with very rare SNPs with minor allele frequency lower than 1%). Indeed, using less stringent cut-off p-values values for calculating the polygenic risk score increases the predictability, which shows that many non-statistically significant GWAS hits do have predictive value indicating the presence of SNPs with small effects that did not get picked up with current sample sizes (Ripke et al. 2013). Furthermore, Ripke and colleagues estimated that 8300 common independent SNPs contribute to the estimated heritability of 50%. Alternatively, epistasis (interactions between genetic effects) could also explain (at least in part) the missing heritability, as we do not yet have the statistical knowledge to test for associations between all genes. If epistasis is indeed present, the 81% heritability by twin and family studies might be an overestimation (Zuk et al. 2012). Despite

the fact that we do not know the exact underlying genetic mechanisms, GWA studies have confirmed that schizophrenia is highly polygenic and genetically very complex.

To understand the biological and phenotypic effect of the associated SNPs, results from GWA studies should be verified by functional follow up studies. However, the hits are often in intergenic regions of the DNA, and are thought to regulate the expression of nearby genes, but it is uncertain which genes will be affected by these intronic regions. Therefore, analysis of such variants would imply a comparison of hundreds, if not thousands of downstream effects with small effect sizes, again requiring large sample sizes. This makes the use of GWAS to find the actual causal genes less straightforward. Biostatistical analysis of the GWAS hit should provide more insight in the underlying causal mechanisms in SCZ by converging genetic information into specific pathways. Gene ontology analysis in these hits has already given us mechanistic clues (such as glutamatergic neurons and calcium signaling; (Consortium 2014)) and more complex biostatistical tools have been developed to interpret this type of data more easily (Watanabe et al. 2017). However, these types of analysis still miss information as we have not yet tackled the entire genetic background of SCZ and as we still do not fully understand the complexity of the effects on intronic regions on gene expression. In conclusion, GWAS provided a powerful method to identify genetic variants that contribute to the increased risk to develop schizophrenia, although we need further research and additional tools to analyze the full impact of these SNPs on heritability, biological mechanisms and clinical presentation.

Glial component

The pathological pathways involved in SCZ have long been hypothesized to mainly affect neurons. Accordingly, the vast majority of research conducted in SCZ has been focused on synapses and neurons. However, there is enough evidence suggesting a significant role for glia cells (astrocytes, oligodendrocytes and microglia). Gene expression abnormalities in all types of glia has been linked to SCZ (Bernstein et al. 2015). Goudriaan et al showed that astrocyte and oligodendrocyte gene sets are associated with an increased risk of developing SCZ (Goudriaan et al. 2014). White matter differences have been reported (Friston 1998; Kelly et al. 2018; Dietz et al. 2020) and they can proceed clinical symptoms (Samartzis et al. 2014). The white matter in the subcortical frontal regions of the brains of patients seems to be affected in particular, as analyzed with diffusion tensor imaging (DTI) (Shergill et al. 2007; Karlsgodt et al. 2008). Furthermore, the reduced fractional anisotropy seen in the frontoparietal network have been shown to correlate with working memory deficits in patients (Shergill et al. 2007). The severity of the reduction in fractional anisotropy correlates with disease severity (Sun et al. 2015). Administration of antipsychotic medication has been shown to increase fractional anisotropy in several regions, showing a further association between connectivity abnormalities and SCZ (Ozcelik-Eroglu et al. 2014). Dietz, Golmand

and Nedergaard (Dietz et al. 2020) hypothesized that activation of microglia in the prenatal period (by infectious diseases or chronic stress) can lead to deficits in glial maturation. In turn, this leads to altered astrocytes that cannot buffer potassium properly and are also less efficient in clearing glutamate from the extracellular space. Interestingly, given the function of glia cells in the brain (especially for astrocytes and oligodendrocytes), they could very well underlie the hypotheses described above, as they are indispensable in normal synaptic transmission, synchronization of neurons and (long range) connectivity between brain regions. The involvement of astrocytes and oligodendrocytes in SCZ will be further discussed in Chapters 6 and 7.

1.3 - HYPOTHESES IN SCZ

Dopamine

Throughout the past century, several hypotheses have been developed to explain SCZ. A very prominent hypothesis in the etiology of SCZ is the dopamine hypothesis, suggesting schizophrenia patients have increased dopamine levels in their striatum. This hypothesis is based on the fact that most antipsychotic medications act through blockade of the dopaminergic D2 receptors on the presynapse (Howes and Kapur 2009). Specially the typical antipsychotics have high affinity for the D2-receptores, but even the new generation antipsychotics, which directly affects the serotonergic system, still bind to the D2 receptors. Moreover, the effects of most antipsychotics become apparent when the levels of the drug in the CNS is sufficient to occupy 70% of the D2 receptors (Uchida et al. 2011). In addition, the baseline occupancy of the D2 receptors by dopamine predicts the response to treatment on the positive symptoms (Abi-Dargham et al. 2000). More direct evidence for a role of dopamine in SCZ is provided by the finding that increased dopamine production has indeed been seen in the prodromal phase and in first-episode psychosis. This increase was most prominent in the corticostriatal systems, which is involved in estimating the probability that a stimulus is rewarded (Stone et al. 2010). It is hypothesized that this explains paranoid thoughts in schizophrenia as the increase in dopamine may lead to a higher sense of salience of otherwise innocuous stimuli (Cannon 2015). The dopamine theory has been challenged by reports showing a lag of 2 to 4 weeks between the D2 blockage peak and amelioration of symptoms after medical intervention (Howes and Kapur 2009). Since the dopaminergic system is of course not an isolated system within the brain, this lag could indicate that the true effect of the antipsychotic drugs is due to downstream effects. Despite the evidence for the involvement of dopamine in SCZ pathology, the role of dopamine could be linked to other neurotransmitters.

GABA

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain. GABAergic neurons are also thought to underlie SCZ pathology. Dopamine strongly interacts with glutamate and GABA to modulate cortical circuits (Lewis and Sweet 2009). Dopaminergic presynaptic terminals in the striatum and frontal cortex are controlled by intrinsic inhibitory GABAergic neurons as these presynaptic terminal express $GABA_B$ receptors. The GABAergic neurons receive glutamatergic input through N-methyl-D-aspartate (NMDA) receptors, which subsequently determine the level of inhibition on the dopaminergic neurons (Javitt et al. 2005). In other words, GABAergic cells receiving less glutamatergic input will lead to increased dopaminergic levels. This is in line with the findings that cortical GABAergic neurons control dopamine release in the striatum (Karreman and Moghaddam 1996). Therefore, researchers also study the role of glutamatergic and GABAergic neurons in SCZ pathology, in specific NMDA receptors.

NMDA receptors and glutamate

NMDA hypofunction is another well-known and well-studied hypothesis in the field. NMDA antagonists, such as ketamine and phenylcyclohexylpiperidine (PCP), rapidly induce schizophrenia-like symptoms, including positive, negative and cognitive symptoms (LUBY et al. 1962; Javitt and Zukin 1991), which formed the basis for this hypothesis. Several researchers claim that mainly GABAergic interneurons are affected by the NMDA hypofunction due to their high expression of NMDARs and their dependency on this receptor. Low concentrations of glutamate in the anterior cingulate cortex (ACC) lead to lesser stimulation of the GABAergic neurons, which in turn leads to disinhibition in the striatum, i.e. increasing dopamine levels. Furthermore, amphetamine (which inhibits NMDARs and thus leads to NMDAR hypofunction) leads to elevated dopamine levels in the striatum and to SCZ-like symptoms (Nakao et al. 2019). In line with this hypothesis, there is considerable evidence for a correlation between glutamate concentration in the ACC and symptom severity, psychosis, worse neuropsychological performance and decreased chance of remittance (Dempster et al. 2015; Egerton et al. 2018), although reports on glutamate concentrations in the medial prefrontal cortex (PFC, including the ACC) vary (Poels et al. 2014; Jauhar et al. 2018). Furthermore, Jauhar et al showed that there is a correlation between the striatal dopamine synthesis capacity and low glutamate concentration in the ACC in first episode patients. Interestingly increases in glutamine and glutamate (Glx) correlate with symptoms depending on the brain region: frontal and temporal elevation correlate with auditory hallucinations (Hugdahl et al. 2015) and inferior parietal white matter elevations correlate with psychotic exacerbations and symptom severity (Ota et al. 2012). Furthermore, medication normalizes or even further decreases Glx levels (Gallinat et al. 2016). Although several studies indicated decreased glutamate levels in the brains of patients, it remains a matter of debate what the

effects are of such alterations in activation of the NMDARs in interneurons. One proposed consequence is a dysconnectivity and excitation/inhibition imbalance discussed below.

Dysconnectivity

In line with the pioneering work of the researchers discussed in the history section, abnormal brain connectivity is one of the main hypotheses in schizophrenia. Modern technology, such as diffusion tensor imaging and functional connectivity using functional magnetic resonance imaging (fMRI) and electro-encephalography (EEG), has mainly confirmed their d**y**sconnectivity **(altered connectivity)** theory in schizophrenia (Collin et al. 2016; Lanillos et al. 2020). Although increased connectivity has been reported in SCZ, the majority of researchers report d**i**sconnectivity **(decreased connectivity)**(Collin et al. 2016). The first evidence for the disconnectivity theory was published in the 80's and gained support over decades of SCZ research as electro-encephalogram (EEG) abnormalities, reduced white matter volume and reduced white matter integrity were shown by MRI and diffusion tensor imaging (DTI). The idea behind the dysconnectivity hypothesis is that it is not accountable by one single brain region but it is reduced interaction between several regions (Friston 1998; Lanillos et al. 2020). Especially the hippocampus and the PFC have repeatedly been shown to have functional and structural abnormalities (Sigurdsson 2016). Dysconnectivity can be caused by excitation/inhibition imbalance, which has also been hypothesized to be the causal pathway in SCZ. Electroencephalography (EEG) indicated disinhibition in rest and in stimulus evoked gamma-bands of SCZ patients (Gonzalez-Burgos et al. 2010; Gonzalez-Burgos and Lewis 2012). Delta and gamma hyperconnectivity are most prominent in early phase patients (Di Lorenzo et al. 2015), while alpha oscillation decrease is independent of disease duration (Lehmann et al. 2014; Di Lorenzo et al. 2015). As summarized by Foss-Feig et al. (2017), SCZ patients also have difficulty sensory gating, they have abnormal spreading of excitatory activity, impaired high frequency oscillations and failure to downregulate task-irrelevant activity. Moreover, EEG during auditory tasks further indicates GABAergic dysregulation (Foss-Feig et al. 2017) and alteration in NMDAR function may underlie these findings. Functional MRI supports the findings of dysconnectivity, and reflects the proton magnetic resonance spectroscopy as higher glutamate levels are associated with striatal and thalamic connectivity (Foss-Feig et al. 2017). Hyperconnectivity of the PFC has been reported in unmedicated patients, and medication normalized this connectivity, which correlates with improvement of symptoms (Anticevic et al. 2015). Higher stimulus response was seen after auditory stimulation which correlated with glutamate levels (Falkenberg et al. 2012). One potential underlying cause for the dysconnectivity is thought to be the decreased number of synapses that has been frequently reported for SCZ. This decrease in synapse counts could be due to excessive pruning (Hoffman and Dobscha 1989), which happens during adolescence (Huttenlocher 1979) and coincides with the typical onset of the disorder. In post mortem tissue of SCZ patients, it is also shown that microcircuits in the

cortex are altered (Marder and Cannon 2019) and there are lower counts of dendrites and dendritic spines in the prefrontal and parahippocampal regions (Glausier and Lewis 2013; Konopaske et al. 2014), in line with a decrease in grey matter in these regions. Therefore, it is tempting to hypothesize that low scale reductions in connectivity (i.e. lower spine densities or white matter abnormalities) eventually lead to decreased macroscale connectivity (i.e. functional connectivity). Although this might be wrongful, as there could be processes such as disinhibition and new connections leading to enhanced functional connectivity ((Collin et al. 2016). Taken together, several studies have shown abnormal connectivity between different brain regions that may underlie different symptoms. Moreover, several SCZ associated malfunctions (NMDA hypofunction, dopamine hyperfunction, excess glutamate and altered synapse number) can lead to dysconnectivity, suggesting this may be a point of convergence in SCZ pathology.

1.4 - MODELING SCZ

Mouse models

Much SCZ research has been performed on patients, which has given researchers many clues on the basis of this pathology. This type of research is limited to non-invasive methods, which does not allow the investigation of phenotypic differences at cellular and molecular level. Such phenotypic differences are often investigated on postmortem human tissue, as living human brain materials is sparse. However, human studies (both patient studies and postmortem studies) are confounded by many different factors such as medication and drug use, which means that these findings might not underlie SCZ (Sigurdsson 2016). Therefore, the use of animal models has been indispensable in the field of neuroscience for decades, and they have formed the basis of our current knowledge of molecular pathways and genetic effects. Many different animal models for SCZ have been developed that are based on different types of validity. First, there are models based on known genetic and environmental factors. Such models are considered to have high construct validity (Sigurdsson 2016). Secondly, there are models that reconstruct findings of human studies, such as increased dopamine receptor expression. Almost all models of SCZ have face validity, meaning that animals show some behavioral signs of SCZ. In the current section I will highlight the most prominent neuronal deficits that are found in SCZ mouse models in relation to the hypotheses discussed above.

Neurotransmitter imbalance

As the dopamine hypothesis is one of the most prominent hypotheses in the field of SCZ, this neurotransmitter has extensively been investigated in mouse models. These models have indeed shown increased activity in dopaminergic ventral tegmental area (VTA)

neurons (maternal immune activation model) which was due to hyperactivity in the ventral hippocampus (Lodge and Grace, 2009). This is thought to result in locomotor hyperactivity seen in this model and their altered reward signaling. The latter is thought to underlie psychosis as described earlier in this introduction. Moreover, increased sensitivity to auditory stimuli in the thalamus of the 22q11.2 model has been shown to arise from D2-receptor overexpression, which is thought to underlie the PPI deficits (Chung et al. 2014). Maternal immune activation and neonatal ventral hippocampal lesion models affect the dopaminergic modulation of the PFC, where PFC neurons respond with excitation rather than inhibition to stimulation from the VTA (Sigurdsson 2016). Interestingly, DISC1 neurons from the PFC do not show the inhibitory response to D2 antagonists. These results are in line with the thought that dopamine signaling is altered in patients and that the interplay between dopamine, GABA and glutamate is altered (Sigurdsson 2016). Moreover, these animal models provide further molecular insight into how these alterations might occur.

Dysconnectivity

As dysconnectivity is one of the earliest hypotheses in SCZ pathology and has received much support from clinical data, this hypothesis also received much attention in several animal models. Researchers found that there is indeed altered connectivity between and within several brain regions in many different models of SCZ. Local synchrony alterations are found in NMDAR hypofunction models, which including increased gamma oscillations (Kocsis et al 2013). These gamma alterations have also been seen in genetically engineered models including *Neuregulin 1* and 15q13.3 mice. However, results regarding gamma oscillations are inconsistent as other models tend to show the opposite or no difference (Sigurdsson 2016). Therefore, mouse models seem inconsistent in the replication of altered gamma oscillations and more research would be required to determine the role of these oscillations in SCZ.

As discussed earlier in this introduction, patients also show deficits in long range synchrony and this has been consistently replicated in several mouse models of SCZ. Especially the synchrony between hippocampus and the PFC has been studied, which manifests as decreased coherence between the local field potentials in these two brain regions or reduced phase locking between neurons from the two structures (Sigurdsson 2016). Interestingly, the reductions in synchrony correlate to behavioural impairments and it is found in almost all mouse models of SCZ. Synchrony alterations are not limited to hippocampus and PFC, but have also been shown between motor and sensory regions, PFC and thalamus and the left and right hippocampi. In many cases, the long-range connectivity is altered without obvious abnormalities in local neuronal synchrony, and specifically long-range connections have shown to be altered using DTI in patients. The *Zdhhc8* mouse model shows that hippocampal neurons projecting to the PFC indeed have less branches, although the altered long-range

connectivity could also reflect alterations in plasticity (Pettersson-Yeo et al. 2011). The long-range dysconnectivity seems to be in line with patient data as described above. Furthermore, mouse models allow the investigation of the origin of such dysconnectivity at lower scale, such as the plasticity at the synaptic level, which may underlie (functional) dysconnectivity seen in animal models and patients. Short term plasticity is altered in several SCZ mouse models (both long and short term, including models: 22q11.2, *Disrupted In Schizophrenia 1*, *NRG1*, calcineurin) in PFC, hippocampus and auditory cortices. Long term plasticity has been shown to be altered in 22q11.2, *DISC1*, *NRG1* in PFC and hippocampus, Sigurdsson 2016). Some of them report alterations in long term potentiation plasticity while others show alterations in long term depression. According to Sigurdson at al., these alterations are likely not a result of affected basic synaptic transmission, as most studies did not find such abnormalities. In summary, there is strong support for an altered connectivity in SCZ cases coming from both human patient studies and from mouse models.

Another potential underlying cause for dysconnectivity is E/I imbalance, which also has been shown by several reports as discussed above. In accordance, several SCZ mouse models have been shown to have altered E/I balance. Indeed, a reduction of parvalbumin (PV) interneurons was shown (Niwa et al., 2010), but also a reduction of inhibitory neurons output which was sometimes accompanied by an increase in excitatory transmission has been reported in SCZ mouse models, reflecting E/I imbalance seen in human subjects. E/I imbalance has been shown within local circuits, but also across different brain regions such as the connection between the ventral tegmental area (VTA) and the cortex. Such findings have been found in genetically engineered mouse models (*DISC1, NRG1*, D2-receptor overexpression, 15q13.3) but also in models induced by neonatal stress and NMDA antagonists (Sigurdsson 2016). The underlying cause of this dysconnectivity remains elusive despite the possibility to investigate cellular and molecular phenotypes in mouse models, as the difference models contradict each other. The latter can reflect the heterogeneity in SCZ or the fact that these underlying causes for the dysconnectivity are not the causal in SCZ. Moreover, an E/I imbalance is supported by the data obtained from a large variety of models, supporting the idea that this might underlie dysconnectivity in patients.

Limitations of mouse models:

However, the use of animal models is also limited by several factors: there are ethical concerns and the translation to the human condition can be very difficult, as both species are clearly different. Although there are endophenotypes that are constant between patients and animals, such as prepulse inhibition (PPI) (Sigurdsson 2016), positive symptoms are much harder to model in mouse models, restricting their face and predictive validity (O'T et al., 2015). Genetic models are often limited by the possibility of compensatory mechanisms, redundancy and risk for lethality. Moreover, knockout mouse models are generally not

representative since SCZ often does not harbor such genetic variations. Translational limitations due to poorly conserved non-coding regions and neuronal networks also pose important drawbacks of animal models.

Human pluripotent stem cells

The research performed by Shinya Yamanaka which has been awarded with the Nobel Prize in 2012, provides a solution to this problem. In their research, Yamanaka showed that the ectopic introduction of 4 transcription factors (OCT3/4, SOX2, KLF4 and c-MYC; now known as the Yamanaka factors) can reprogram a somatic cell back into a pluripotent stem cell stage seen during the embryonic phase by reorganizing the epigenome. These cells are called induced pluripotent stem cells (iPSCs) and they are genetically identical to the donor. iPSCs have the same properties as embryonic stem cells. They are capable of replicating themselves, and have the potential of becoming every type of cell of the human body but are not capable of forming an organism when implanted into the uterus. Following 1998, when the first embryonic stem cells (ESCs) for *in vitro* use were derived from the inner cell mass of an embryo, the prospects of human *in vitro* models expanded and accelerated upon the introduction of iPSCs. Stem cells form a great tool for neuroscientists, as this opens the door for molecular analysis in human tissue that is usually unavailable, such as the brain. After the development of differentiation protocols, allowing stem cells to become cells present in the human brain (such as neurons, astrocytes, oligodendrocytes and microglia), much research has been performed using these human cells. They gave us insight in molecular pathways involved in the human brain development and diseases. Human iPSCs provides clear advantages over ESCs by capturing the complex genetic components in psychiatric disorders, involving hundreds or even thousands of genes that still remain mainly unidentified. This is hard to model by gene editing which is often used when modeling monogenic disorders. This way, the complex architecture of many psychiatric traits remained for long unaccounted for. By selecting patients, the genetically complex architecture of psychiatric disorders can be maintained *in vitro*. For this reason, iPSCs have been increasingly used in the field of neuropsychiatry.

1.5 – THESIS AIM AND LAYOUT

The main aim of the current thesis is to determine the role of astrocytes in SCZ pathology using a human *in vitro* research model based on iPSC technology. In order to obtain such a model, we have considered several aspects that are crucial in SCZ pathology and in iPSC models. These aspects will be discussed in the chapters 2-4, after which we will discuss our SCZ-related findings in chapter 5 and 6. Chapter 7 consists of a general discussion

concerning all chapter in this thesis and future perspectives. The overall aim of this thesis is summarized in Figure 2.

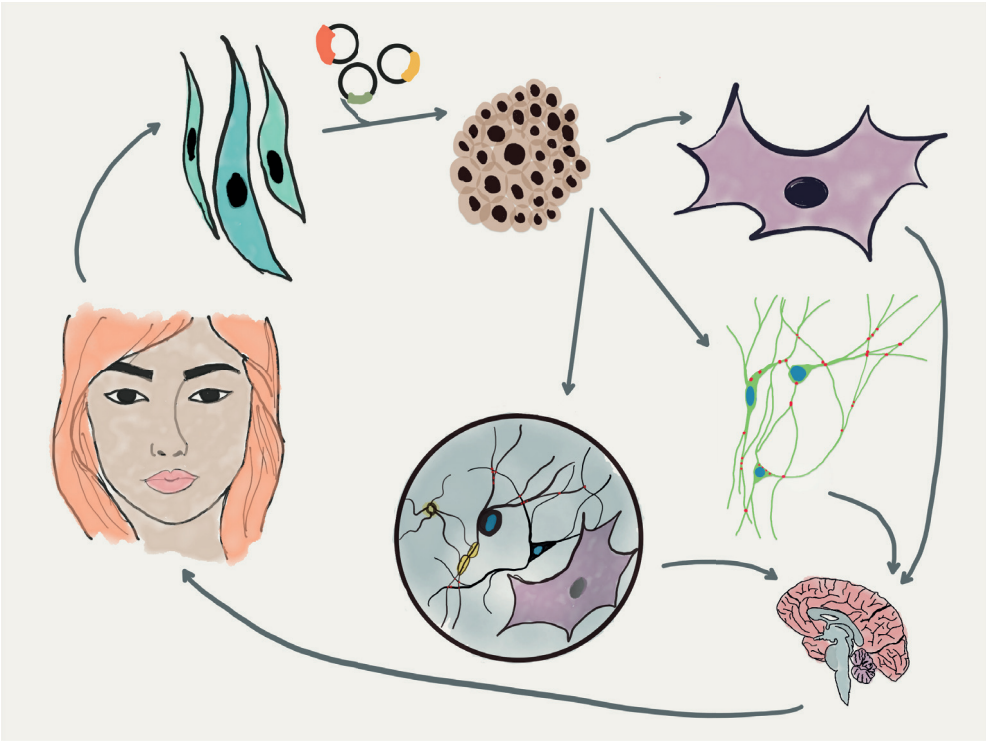


Figure 2: Illustration of thesis aim: fibroblasts of cases and controls were collected and reprogrammed episomaly to prevent integration of the reprogramming factors and thereby disturbance of potential SCZ-associated genes. iPSCs were differentiated into neurons, astrocytes and brain organoids in order to detect differences between cases and controls. Eventually we aim to translate these differences to the human brain and human behavior.

Genetic complexity – Chapter 2

SCZ is highly polygenic and arises through the synergistic effects of hundreds (if not thousands) of genetic variants. Associated SNPs are common and have low risk, while CNVs are rare and convey a relatively large risk. This leads to heterogeneity between patients which in turn negatively affects statistical power to detect disease associated phenotypes. This is particularly problematic in high cost, laborious experiments, where the increase of sample sizes is not straightforward, as is the case in iPSC research. We discuss this aspect in Chapter 2, where we explain the genetic background of SCZ in relation to sample sizes and variability. In this chapter, we discuss the complications of studying CNVs and selecting

patients only based on clinical diagnosis, and we propose a study outline that allows the investigation of genetic variants in such a way that allows claims of causality. Moreover, we discuss the use of polygenic risk scores (PRS) for the stratification of subject selection in order to obtain higher statistical power. This chapter explains the rationale behind our choice of participants in our SCZ research.

Reprogramming – Chapter 3

To generate a SCZ model that reflects the genetic complexity of this disorder, we will generate iPSC-derived astrocytes from high PRS cases, cases carrying a SCZ associated CNV and low PRS controls. For the reprogramming into iPSCs, one must introduce the reprogramming factors into the donor fibroblasts. There are several methods to reprogram somatic cells such as fibroblasts, each posing its advantages and disadvantages. Two well-known and frequently used procedures are the lentiviral and episomal reprogramming. As the first method integrates the ectopic transcription factors into the host genome, this generates a stable expression of these factors. On the other hand, this leads to the disruption of the host genome, which can vary depending on the integration site. This could lead to the disruption of a SCZ-associated gene, and potentially to increased variability as the integration site can alter between lines. Episomal reprogramming does not integrate, which means that the expression of the factors is not as stable as in the lentiviral reprogramming. In Chapter 3 we investigated the differences between these two reprogramming methods in order to choose the most suitable method for the generation of our iPSC lines.

Variability in iPSC research – Chapter 4

The field of iPSCs is relatively new, and therefore still lacks standardization. In literature, we see that different reports use different amounts of donor and of clones per donor. Moreover, the hierarchical clustering between clones and donors differs per study and might be due to (i) reprogramming methods, (ii) differentiation protocol and (iii) interlaboratory differences as some reports include iPSC lines from different labs. Moreover, the majority of reports prior to this chapter have investigated variability between clones and donors at stem cell stage only and all reports have done this based on transcriptome analysis which was accompanied by functional analysis in some (electrophysiology). None of the reports have used proteome data to compare the variability between clones and donors, that were generated in parallel by one single researcher, as we have done in Chapter 4. In these experiments, we have used our astrocyte differentiation protocol to generate astrocytes from multiple clones from multiple healthy donors. This gave us insight into the variability of our donors, clones, reprogramming protocol, and differentiation protocol, which allowed us to further optimize our study design to obtain high statistical power without neglecting the limitations in sample size that is posed by iPSC research.

Astrocytes in SCZ – Chapter 5

Lastly, after performing several experiments to determine the best study design for our SCZ research, we have used the results presented in the previous chapters to create a robust model for SCZ with its genetic complexity using iPSCs. This included the strategic selection of patients and controls based on genetic information and the selection of more donors rather than more clones. In Chapter 6, we have compared the astrocytes from SCZ cases to the ones generated from controls at morphological and transcriptional level, which revealed 10 differently expressed genes between SCZ and controls. We further validated the expression of our most significant hit and we have investigated potential functional consequences thereof. More specifically, we have investigated potential in glial or neuronal differentiation skewing, we have performed western blots, overexpression experiments and neuron-astrocyte co-cultures in order to investigate potential alterations in network formation between neurons co-cultured with SCZ and control astrocytes. This study reveals a potential new candidate gene in SCZ-pathology.

3D culturing – Chapter 6

As already discussed above, myelination has been shown to be affected in SCZ patients. Using the ideal approach as outlined in the previous chapters, we have attempted to model white matter alterations in SCZ using our high PRS cases and low PRS controls. However, 2 dimensional cultures as do not mimic the complex architecture of the brain. Moreover, to properly asses the myelinating potential of oligodendrocytes and the interplay between neurons, astrocytes and oligodendrocytes, a more complex model is required. This should include the third spatial dimension, and the variety of cell types one is willing to investigate. In Chapter 6, we therefore generated brain organoids from a subset of patient lines, in order to look for glial defects after the inclusion of multiple cell types and a third dimension.

Discussion – Chapter 7

In the current thesis, we develop an optimized study design for the *in vitro* modeling of genetically complex disorders, considering genetic complexity, variability and statistical power. In Chapter 7 I will discuss the results found in the current thesis and I will put these results in a broader perspective based on other literature.

Table1: Overview of findings in animal models

Model	**Structural phenotype**
Gestational MAM	Reduced PFC and hipppocampal size, reduced soma size hippocampus, enhanced nACC DA release, hyperactive VTA neurons (DA), decreased PV interneurons in limbic and cortical areas
Post weaning social isolation	Reduced PFC volume, reduced dendritic spine density, altered cytoskeleton, loss of PV interneurons, loss of Reelin in hippocampus, reduced D1 binding in PFC, hyperactive VTA neurons (DA), increase striatal D2 high
Amphetamine	Enhanced mesolimbic DA response, altered acethylcholine function in PFC,
PCP	Reduced DA and glutamine release in PFC, reduced spine density in FC neurons, reduced spine density in PV neurons in PFC and hippocampus
Ventral hippocampal lesion	Enhances DA release from nACC upon stress, reduced NAA level in PFC, reduced GAD67 mRNA
DISC 1 KO	Reduced brain volume and cortical thickness, enlagerged ventricles, reduced dendritic desnity in hippocampus and PFC, reduce PV in hippocampus in some mutants.
Neuregulin1 and ErB4 KO	Enlarged ventricles, reduced hippocampal dendritic spine density, reduced functional NMDAR in FC.
Dysbindin KO	Hyperexcitability in pyramidel neurons of PFC, altered structure and formation of synapses, disrupted DA signalling (D2) and altered neuronal excitability
Reelin KO	Increased neuronal packing, decreased dendritic spine density in PFC and hippocampus.
MIA	Altered PV interneurons and their associated perineuronal nets. Decreased Complex I and ATP production
22q11.2 del	Cortical and subcortical grey matter volume alterations, reduced spine size, reduced dendritic complexity

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde

R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Chun Sungkun, Westmoreland Joby J, Bayazitov Ildar T, Eddins Donnie, Pani Amar K, Smeyne Richard J, Jing Yu, Blundon Jay A, Zakharenko Stanislav S (2014) Specific disruption of thalamic inputs to the auditory cortex in schizophrenia models. Science 344(6188):1178–1182.

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380. e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen

FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J, White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical region in humans and the region of conserved synteny in mice: a search for candidate genes

at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism

and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity . Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684 . https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic

JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of "Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

Lodge Daniel J, Behrens Margarita M, Grace Anthony A (2009) A loss of parvalbumin- containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia. J Neurosci 29(8):2344–2354.

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

McGrath JJ, Petersen L, Agerbo E, Mors O, Mortensen PB, Pedersen CB (2014). A Comprehensive Assessment of Parental Age and Psychiatric Disorders. JAMA Psychiatry. 71(3):301–309. doi:10.1001/jamapsychiatry.2013.4081

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Niwa Minae, Kamiya Atsushi, Murai Rina, Kubo Ken-Ichiro, Gruber Aaron J, Tomita Kenji, Lingling Lu, et al. (2010) Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. Neuron 65(4):480–489.

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pettersson-Yeo William, Allen Paul, Benetti Stefania, McGuire Philip, Mechelli Andrea (2011) Dysconnectivity in schizophrenia: where are we now? Neurosci Biobehav Rev 35(5):1110–1124.

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe

C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B, Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

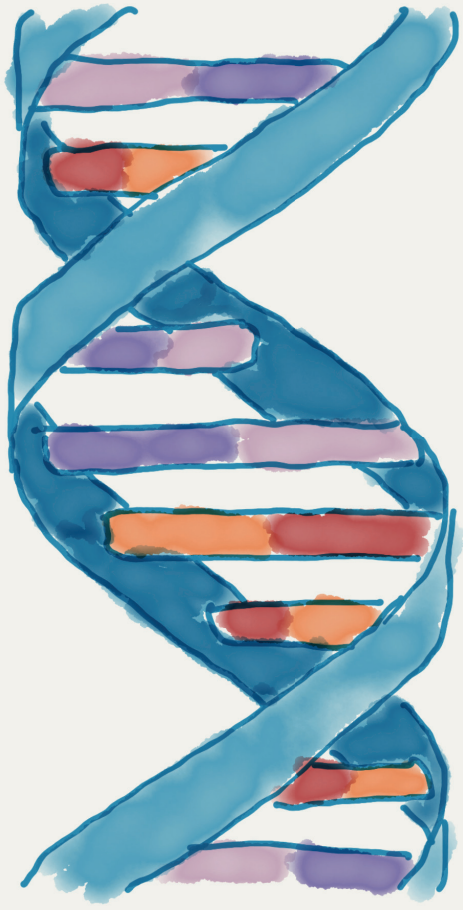
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Chapter 2

Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity

Stephanie D. Hoekstra,[1] Sven Stringer,[1] Vivi M. Heine,[1,2] and Danielle Posthuma[1,3,*]

[1]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, Netherlands
[2]Department Pediatrics/Child Neurology, VU University Medical Center, Amsterdam, Netherlands
[3]Department of Clinical Genetics, VU University Medical Center, Amsterdam Neuroscience, Amsterdam, Netherlands

Published online 2017 Jun 13. Front Cell Neurosci. 2017; 11: 164.

ABSTRACT

Induced pluripotent stem cell (iPSC) technology is more and more used for the study of genetically complex human disease but is challenged by variability, sample size and polygenicity. We discuss studies involving iPSC-derived neurons from patients with Schizophrenia (SCZ), to exemplify that heterogeneity in sampling strategy complicate the detection of disease mechanisms. We offer a solution to controlling variability within and between iPSC studies by using specific patient selection strategies.

Keywords

IPSC, schizophrenia, variability, disease models, genetics, psychiatric diseases, statistical power, stem cells

INTRODUCTION

Induced pluripotent stem cells (iPSCs) are increasingly being used to investigate disease mechanisms underlying complex diseases, like schizophrenia (SCZ), autism spectrum disorders and major depressive disorder. The genetic architecture of complex diseases is characterized by its polygenic nature, with thousands of genetic loci increasing disease risk, and by various combinations of risk loci carried by different patients. Such genetic heterogeneity may have undesirable effects on the outcomes and the interpretations of iPSC studies. When genetic heterogeneity is not controlled and participants in iPSC studies are e.g., selected based on the presence or absence of a polygenic disease, the cases may have partly or even completely different risk alleles that contribute to the disease. Especially since iPSC studies typically involve few participants (<30), an unlucky draw of cases (yet the same holds for controls) may result in genetically heterogeneous cases (and controls). If such genetic heterogeneity is related to heterogeneity at the cellular level, variability at a biological read-out will increase, which will in turn decrease the statistical power to detect a difference in the biological read-out between cases and controls. Here we will discuss the importance of addressing genetic heterogeneity and patient selection strategies in the design of iPSC studies for complex disorders.

Heterogeneity and statistical power

When genetic heterogeneity is not controlled, differences in biological read-out seen between cases and controls in study 1 may be not be found in study 2. This can reflect a false positive finding in study 1, but may also reflect genetic heterogeneity between studies. This is unfortunate, as replication is important and will solidify the conclusions of a study.

To illustrate how the polygenic background of complex disorders affects the statistical power of iPSC studies, we calculated the effect of variability (induced by genetic heterogeneity) in the biological readout on the power to detect statistically significant differences in the readout between cases and controls (Figure 1). The results presented in Figure1 are based on a power analysis in which we assume a design with two contrast groups (e.g., case vs. control) and a continuous outcome measure (e.g., expression of proteins of interest). Heterogeneity between cells of different subjects within each group is expressed in standard deviations (sd). Without loss of generality we define the relative heterogeneity as the ratio between the within-group standard deviation and the mean difference between the groups. If the mean difference is 1, this measure of heterogeneity is simply the standard deviation in outcome within each group. Figure 1 shows how large the variability within a group is relative to the observed mean difference between the groups. Thus the larger this heterogeneity, the larger the required sample size becomes. Ideally the variability within each group is much smaller than the variability between the groups. On the other hand, when the relative heterogeneity is

large, say 1.2, the standard deviation is 20% points larger than that of the observed average group difference. In this case it would be difficult to detect a significant difference between groups. Figure1 shows that with samples sizes around 5 the optimal ratio is 0.5. However, since we cannot control effect sizes of the biological read-out (i.e., the difference in the measured cellular phenotype between cases and controls), it would be advisable to reduce variability by reducing genetic heterogeneity within one group.

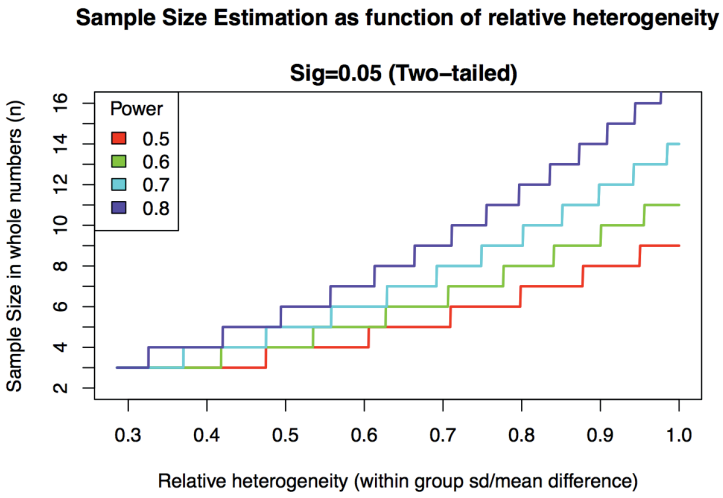


Figure 1: Sample size increase as a function of relative heterogeneity for different levels of statistical power. Relative heterogeneity is defined here as the ratio between within-group standard deviation and mean group difference.

One way to increase statistical power is to increase sample size. This would make unlucky draws less likely. However, due to the current labor-intensive nature of iPSC studies, sample sizes above 10–30 individuals are often not feasible, and alternative strategies are needed. One such strategy is to use genetically-informed decisions in patient (and control) selection. By selecting genetically homogeneous cases and controls, within-group variance can be reduced, which is a critical determinant in both increasing statistical power and evaluating results from iPSC studies for complex disease (Figure1).

SCHIZOPHRENIA

To illustrate the importance of reducing genetic heterogeneity we discuss several examples in the context of (SCZ), a complex disorder (SchizophreniaWorking Group of the Psychiatric Genomics Consortium et al., 2015) for which already a number of iPSC-based studies have been published and in which a number of different patient selection strategies have been applied.

Table 1 lists current iPSC studies investigating SCZ with their selection of patients and controls. To illustrate differences in patient selection between studies we also list Odds Ratios (ORs) and penetrance for SCZ and other disorders. As shown in this table, several studies selected cases for the presence of a specific SCZ-associated genetic component of large effect. These studies selected specific genetic variants to reduce genetic and possibly cellular heterogeneity, rather than selecting on diagnosis. Wen et al. (2014), Pak et al. (2015), and Siegert et al. (2015) investigated a single variant in DISC1, NRXN1, and mir137, respectively (also see Table 1). Each of these three studies reported presynaptic deficits in carriers vs. non-carriers, suggesting that these presynaptic deficits are important in the etiology of SCZ. Specifically all three implicated an important role for the release probability of vesicles. The deficit in vesicle release reported by these studies caused a decrease in spontaneous mini excitatory postsynaptic currents (mEPSC) and EPSC amplitude. However, the difference in EPSC amplitude in one study depended on the control taken for comparison (Wen et al., 2014).

Another iPSC study selected SCZ patients with a high likelihood of a genetic burden based on family history rather than carriers of a specific variant, vs. controls that did not have a familial burden. In this study no such presynaptic deficits were reported (Brennand et al., 2011). Comparing these outcomes to the previously mentioned three studies may cast doubt on the presynaptic involvement in SCZ. However, the results from these different studies cannot be directly compared as SCZ is a polygenic disorder and the studies (implicitly) selected patients carrying a variety of risk variants. Different risk factors might affect different cellular pathways that do not lead to presynaptic deficits. Interestingly, Yu et al. (2014), who studied the same patients as Brennand et al. (2011) but only a used a subset of the controls used by the same group (Table1) did find a decrease in mEPSCs frequency and amplitude as reported by the Wen et al. (2014), Pak et al. (2015) and Siegert et al. (2015). The latter underlines the impact of specific patient—control combinations. This idea is further supported by the findings of Wen et al. (2014), who reported that differences in EPSC amplitude were dependent on the chosen control (related or unrelated). Presynaptic deficits might thus still be a causal mechanism in SCZ. The important issue here is that comparison of results between studies assumes that the same contrast groups were used, while this may not always be the case.

Table 1. Summary of SCZ research performed in iPSCs

Reference	genetically informed	patients	Controls	OR	Penetrance	penetrance other	generalization of data	confirmation	SCZ phenotype	other phenotype	potential of bias
Brennand *et al.,* 2011	no	Male early onset SCZ (suicide), brother (SCZ) and sister (SAD), unrelated patient	One commercially availabe new born control (male), 2 males (20-22 y/o) and 4 females (22-25 y/o)	unknown	unknown	unknown	good	No	Low	Low	No
Paulsen *et al.*, 2012	no	Clozapine resistant female patient	H09 (female) line, male control (commercially available fibroblasts)	unknown	unknown	unknown	good	No	Low	Low	No
Robicsek *et al.*, 2013	no	Male with affected mother, female with affected mother, male with affected mother and grandfather, all responding to clozapine	2 controls	unknown	unknown	unknown	good	No	Low	Low	No
Brennand *et al.*, 2014	no	Patients from Brennand et al 2011	One commercially availabe new born control, 1 other male (20 y/o) and 4 females (22-25 y/o) all from Brennand et al 2011	unknown	unknown	unknown	good	No	Low	Low	No
Yu *et al*, 2014	no	Patients from Brennand et al 2011	4 controls from Brennand et al 2011	unknown	unknown	unknown	good	No	Low	Low	No

Hook *et al.,* 2014	no	Male early onset SCZ and two males from Brennand et al 2011	Newborn fibroblasts, 22 y/o female, 25 y/o female	unknown	unknown	unknown	good	No	Low	Low	No
Paulsen *et al.*, 2014	no	Patients from Paulsen et al 2012	controls from Paulsen et al 2012	unknown	unknown	unknown	good	No	Low	Low	No
Hashimo-to-Tori et al 2014	no	Patients from Brennand et al 2011	5 controls from Brennand et al 2011	unknown	unknown	unknown	good	No	Low	Low	No
Wen *et al.*, 2014	yes	Father (MD) and daughter (SCZ), both DISC1 frameshif	Mother/wife of patients, daughter/sister of patients, one unrelated male control	LOD 3.6***	unknown	LOD 7.2***	low	Yes	High	High	Yes
Yoon *et al.*, 2014	yes	3 SCZ patients carrying a 15q11.2 deletion	5 family members	2.15**	2%*	11%*	low	Yes	High	High	Yes
Bundo *et al.,* 2014	yes	2 patients with 22q11.2 deletion	16 y/o female	NA**	12%*	88%*	low	Yes	High	High	Yes
Topol *et al.*, 2015	no	Patients form Brennand et al 2011	6 controls from Brennand et al 2011	unknown	unknown	unknown	good	No	Low	Low	No
Murai *et al.,* 2015	yes	Patients and controls from Wen et al 2014	Mother/wife of patients, daughter/sister of patients, one unrelated male control	LOD 3.6***	unknown	LOD 7.2***	low	Yes	High	High	Yes
Pak *et al*, 2015	yes	Induced NRXN1 deletion and truncation in H01	H01	9.01**	6.4%*	26%*	low	Yes	High	High	Yes

Table 1. Continued

Narla *et al.*, 2017	no	Patients from Brennand et al 2011	One commercially availabe new born control, 1 other male (20 y/o) and 2 females (20-25 y/o) all from Brennand et al 2011	unknown	unknown	unknown	good	No	Low	Low	No
Topol *et al.*, 2016	no/yes	Patients from Brennand et al 2011; 10 additional controls and 10 additional patients, 6 carrying several CNVs	One commercially availabe new born control, 1 other male (20 y/o) and 4 females (22-25 y/o) from Brennand et al 2011, 9 additional controls (5 males, 5 females)	variety	variety	variety	very good	Yes	High/Low	Low	No
Marcatili *et al.*, 2016	no	Clozapine responsive patient	Commercially available control	unknown	unknown	unknown	good	No	Low	Low	No
Marsoner *et al.*, 2016	no	Clozapine resposive patient	None	unknown	unknown	unknown	good	No	Low	Low	No
Siegert *et al.*, 2016	yes	Carriers of SNP in mir-137	Two subjects not carrying the risk SNP in mir137	variety	unknown	unknown	low	Yes	Low	High	Yes
Toyoshima *et al.*, 2016	yes	Patients from Bundo et al 2014	36 y/o female	NA**	12%*	88%*	low	Yes	High	High	Yes
Lin *et al.*, 2016	yes	8 unrelated patients with 22q11.2 deletion (SCZ or SAD)	7 controls	NA**	12%*	88%*	low	Yes	High	High	Yes

In addition, we would like to point out another important issue; in the studies where individuals carried the selected genetic variants, not all individuals were known to be diagnosed with SCZ (Wen et al., 2014; Siegert et al., 2015). If the cells are derived from carriers who do not have the disease and that are passed the age of onset, no definite conclusions can be drawn about the causal role of the reported presynaptic deficits in SCZ, because clearly the presence of the genetic variant as well as the presynaptic deficits do not co-occur with SCZ disease status in all individuals. Causal inferences in the context of SCZ in these studies would have been more reliable when all carriers would have been diagnosed with SCZ. Another important issue is that there seems to be a bias toward variants known to be affecting the synapse. Although SCZ has been hypothesized to be a disease of the synapse, there are studies showing other pathways may have a big impact in SCZ, such as GFAP overexpression and oxidative stress (Paulsen et al. 2012; Robicsek et al., 2013; Toyoshima et al., 2016).

As described above, causality is difficult to claim in complex trait diseases. These studies exemplify that results are (i) highly dependent on the selection of subjects (ii) hard to interpret due to a lack of fully penetrant and disease specific variants. This calls for a more genetically informed selection of patients and controls, to control for genetic background, to improve comparison between studies and to investigate causality.

USING SPECIFIC VARIANTS WITH LARGE EFFECT

Researchers often choose to investigate a specific variant with large effect (mostly CNVs). An important issue when focusing on a single CNV is that many CNVs that have been associated with SCZ have also been associated with other psychiatric disorders such as major depressive disorder or autism (Kirov et al., 2014; Wen et al., 2014; and also mentioned by Pak et al., 2015) and this may occur in the same family. This makes it less likely that these CNVs are SCZ-specific and thus impedes inferences on the specificity of detected cellular phenotypes for SCZ. Thus, both genetic heterogeneity (e.g., people carrying the same rare genetic variant but of completely different polygenic risk) and pleiotropic genetic effects (i.e., the same genetic variant causes multiple diseases) complicate the detection of robust cellular phenotypes that are causally linked to the targeted disorder. Revealing common pathways causal to psychiatric diseases is of great value, and should be investigated further. However, claims of specificity to one particular disease are incorrect when based solely on research to nonspecific genetic background such as CNVs in complex trait genetics. Despite the fact that the role of such variants in other disorders can also be of interest, revealing specific pathways to specific disorders might lead to development of more targeted drugs for specific phenotypes with very little side effects. Furthermore, the fundamental knowledge

of why some individuals carrying the same genetic variant develop SCZ and others develop major depressive disorder will enlighten biological processes as well as genetic ones.

Although selecting for a rare genetic variant of large effect reduces genetic heterogeneity and increases statistical power, rare variants carriers also have a significant predisposition for SCZ caused by common variants (Tansey et al., 2016). This complicates interpretation of results arising from gene editing studies are truly causal to a specific disease. One such example is the study of functional implications of the NRXN1-gene variants (e.g., Pak et al., 2015). NRXN1 is included in a CNV that is one of the most replicated findings for SCZ with Odds Ratio's (ORs) in the order of 9.01 (Kirov et al., 2014). The penetrance of this CNV for SCZ is, however, only 6.4% (Kirov et al., 2014), which means that other causal factors are needed to induce SCZ. Therefore, experimental studies focusing on NRXN1 risk carriers alone may not hold the key to understanding SCZ. The general idea is that patients tend to carry many common risk alleles as well on top of the rare variant associated with SCZ (Tansey et al., 2016). Thus, studies based on gene editing without controlling for genetic background are highly suitable for investigating gene function. However, focusing on a single variant may not always provide sufficient information on cellular pathways involved in SCZ. The use of gene editing could be advantageous when used in combination with high polygenic risk score lines. By introducing a SCZ-associated CNV in lines with high polygenic risk scores an enhanced SCZ phenotype is expected (while in lines with low polygenic risk scores no SCZ phenotypes are expected due to small penetrance).

To claim causality, it is important to include knowledge on the background regarding polygenic risk when selecting patients. The use of polygenic risk scores, especially in combination with the gene editing gives the opportunity of creating continuous variable for risk. This allows correlational analysis between risk and phenotype. If the phenotype correlates with risk score, the probability of a false positive will be very low.

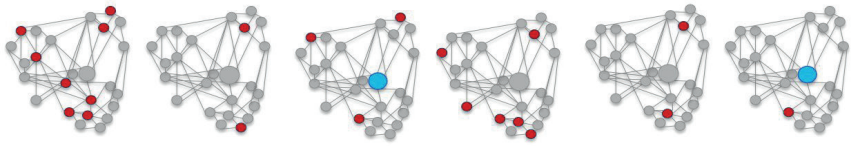
DECREASING HETEROGENEITY BY SELECTING HOMOGENEOUS CASES AND CONTROLS

Studies aiming to reveal causal biological pathways for complex diseases will benefit from improved strategic patient selection, to control for the effects of genetic heterogeneity and pleiotropy. We propose two improvements in patient selection aimed at increasing genetic homogeneity as well as effect sizes: (i) select patients carrying a specific disease-associated genetic variant with a high penetrance and large effect size, or (ii) select patients with high polygenic risk based on common genetic variants. For both strategies the ideal design would be to include four groups of individuals: patients with and without the disease penetrant

variant/high polygenic risk and controls with and without the disease penetrant variant variant/high polygenic risk (Figure 2). This four-way study design allows drawing conclusions on the validity of detected cellular differences for the disease. For example, if suboptimal function of a cellular phenotype is causally related to a disease, the largest phenotype is expected to be most affected in patients with the penetrant variant or high PRS, then in patients without the penetrant variant or low PRS, then in controls with a penetrant variant or high PRS, and lastly in controls without the penetrant variant or low PRS. As described above, many current study designs only include patients carrying known rare variants (with varying penetrance) and controls without the variant, or they choose to include patients and controls solely based on diagnosis. This limits our ability of linking detected phenotypes to the targeted disease. Topol et al. (2016) chose an approach (Table 1) similar to the approach proposed here, i.e., combining patients based on diagnosis only (no SCZ associated CNVs) and patients carrying a variety of SCZ associated CNVs. This design allows the finding of a general phenotype present in both groups of patients. As mentioned before, controlling genetic background by investigating polygenic risk scores allows correlation of the phenotype to genetic burden, reducing the number of potential (no specific) phenotypes and thus decreasing the chance of false positives

DECREASING HETEROGENEITY BY STUDYING FAMILIES

Another way of controlling for genetic heterogeneity by using genetically-informed selection strategies is by using family members, which offers a natural way of matching for genetic background. Family studies have been crucial for understanding the pathology of SCZ, as they offer a model with relatively low genetic variance and therefore high power. Most families affected with SCZ carry a rare variant, with relatively high OR's and relatively large cellular effects as compared to common variants. Due to the genetic relationship any background effects are also partially matched. This is illustrated by the EPSC amplitude reported by Wen et al. (2014) as discussed above. Although rare variants explain only a small percentage of the general SCZ cases, they can be helpful in unraveling cellular pathways involved in SCZ. The presence of a single, relatively large variant with a relatively high penetrance facilitates rescuing of observed phenotypic consequences by gene-editing. However pleiotropic genetic effects may still complicate the interpretation of results and researchers should investigate the presence of other (common) variants present in patients and controls. As seen in Wen et al. (2014) family members carrying the CNV of interest can develop SCZ while other members develop another disease such as major depressive disorder. This is likely the effect of common variants carried by each individual besides the CNV. Also in this setting research will benefit from controlling for genetic background and from reporting on common variants carried by the cases and controls..



Subject	Patient	Patient	Patient	Control	Control	Control
SCZ-associated genotype	high PRS	low PRS	CNV	high PRS	low PRS	CNV
SCZ-specific phenotype	++	+	++	+	--	+
Other phenotype	-	-	++	-	--	++
Study design 1	X	X		X	X	
Study design 2	X		X		X	X

Figure 2: Proposed participant selection strategies. This figure illustrates all types of subjects: subjects with high and low burden carrying only common or also rare variants. Below the schematic representation of each subject one can find a comparison for the two factors: the chance of finding a SCZ associated phenotype and the chance of finding a phenotype associated with other disorders. Two ideal strategies (discussed in the mail text) are also illustrated.

In summary, selecting a genetic variant with high penetrance directly circumvents patient heterogeneity as a confounding factor. If a variant is chosen for its high penetrance and its large effects on risk for the targeted diseases, the effects on a biological phenotype can be expected to be large, thereby increasing detectability and statistical power. In practice however, choosing a single variant may not be straightforward; highly penetrant variants may not (yet) be known for a disease (Falk et al., 2016), or they may be related to other diseases as well (Kirov et al., 2014). If no good genetic candidates are available, the second strategy provides a good alternative. In this selection design, patients and controls are selected with high and low polygenic burden; this strategy is in line with the general assumption that many common variants of small effect converge on a biological pathway or function; i.e., heterogeneity may exist at the level of alleles or affected genes but will be less at the level of biological pathways. This strategy is therefore expected to enhance effect sizes as it involves selection on the accumulated effect of multiple risks. Reducing genetic heterogeneity will

increase the statistical power of studies and will help researchers to overcome a great issue in the stem cell field: sample sizes. As shown in Figure 1, needed sample sizes (assuming 1 iPSC clone per individual) depend on mean differences between groups (effect size) and on the variance within the groups. The use of extremely different cases and controls will help increasing the mean difference between patients and controls, and by ensuring all cases and all controls are genetically matched, genetic heterogeneity within groups will be reduced. This will then lead to an increase in statistical power with smaller sample sizes.

CONCLUSION

The decrease in (genetic and phenotypic) heterogeneity will reduce the number of (nonspecific) phenotypes we observe within and between studies and therefore will increase the chance of finding SCZ-associated causal pathways. In addition, targeted participant selection facilitates comparing results across different studies for replication purposes. As iPSC research is already challenged by variability (Falk et al., 2016), stratification of patient selection as described above to improve statistical power and comparison between studies will therefore be of utmost importance.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication. VMH and DP concaived the study. SDH, VMH, and DP wrote the manuscript, SDH conducted the literature research. SS coducted the statistical power simulations.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

This work was funded by The Netherlands Organization for Scientific Research (NWO VICI 453-14-005). VMH is supported by ZonMw VIDI research grant 91712343 and E-Rare Joint Call Project 9003037601.

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities

across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380.e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J, White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem

cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity . Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684 . https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across

4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of "Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human

Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler

B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B, Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

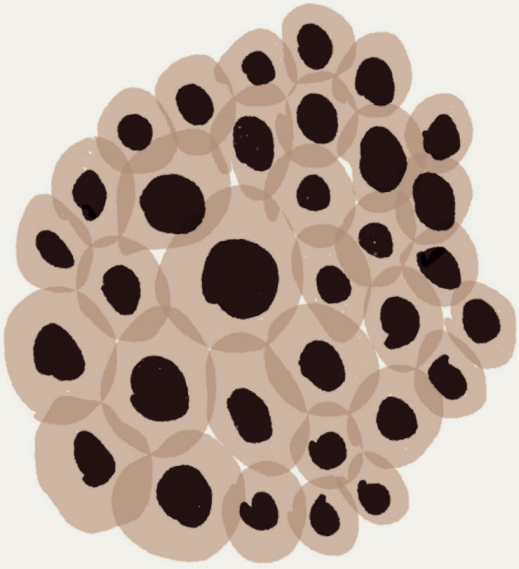
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Chapter 3

Generation of Isogenic Controls for In Vitro Disease Modelling of X-Chromosomal Disorders

Lisa Hinz[1,*], Stephanie D. Hoekstra[1,*], Danielle Posthuma[1,2,#], Vivi M. Heine[1,3,#]

[1]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije Universiteit, Amsterdam, The Netherlands.
[2]Department of Clinical Genetics, VU University Medical Center, Amsterdam Neuroscience, Amsterdam, The Netherlands.
[3]Department of Pediatrics / Child Neurology, VU University Medical Center, Amsterdam Neuroscience, Amsterdam, The Netherlands. * Co-first authors. # Co-senior authors

Published online in 13 November 2018 in Stem Cell Reviews and Reports

ABSTRACT

Generation of proper controls is crucial in induced pluripotent stem cell (iPSC) studies. X-chromosomal disorders offer the potential to develop isogenic controls due to random X-chromosomal inactivation (XCI). However, the generation of such lines is currently hampered by skewed X-inactivation in fibroblast lines and X-chromosomal reactivation (XCR) after reprogramming. Here we describe a method to generate a pure iPSC population with respect to the specific inactivated X-chromosome (Xi). We used fibroblasts from Rett patients, who all have a causal mutation in the X-linked MeCP2 gene. Pre-sorting these fibroblasts followed by episomal reprogramming, allowed us to overcome skewness in fibroblast lines and to retain the X-chromosomal state, which was unpredictable with lentiviral reprogramming. This means that fibroblast pre-sorting followed by episomal reprogramming can be used to reliably generate iPSC lines with specified X-chromosomal phenotype such as Rett syndrome.

INTRODUCTION

Induced pluripotent stem cells (iPSCs) have revolutionized the field of disease modelling, as they provide a platform for functional investigation of polygenic disorders and diseases of unknown genetic cause that are hard to model in traditional transgenic models. While patient material has given much insight into disease pathology, iPSCs provide the possibility to study important developmental periods of disease onset and progression in specific, human cells. Although iPSC technology opened new possibilities to generate in vitro disease models, the field is still challenged by issues like the lack of standardization, low sample numbers and availability of proper controls (Hoekstra *et al.,* 2017; Falk *et al.,* 2016). To generate proper controls that consider genetic background as an important variable, previous study designs have included family members Carvajal-Vergara *et al.,* 2010; Yoon *et al*., 2014) or isogenic controls obtained via gene therapy techniques (Pak *et al.,* 2015). However, family members are not genetically identical and differences in single nucleotide polymorphisms (SNPs) are not accounted for even though the latter can contribute to they can contribute to, or in some cases determine, disease phenotypes (Tansey *et al*., 2016). Furthermore, gene-editing techniques have been shown to have off-target effects (Lin *et al.,* 2014). Therefore, there is still a need for alternative approaches to create well-matched controls in iPSC-based studies.

X-chromosomal disorders form a unique group, as affected female patients have cells with either the mutated or the healthy X-chromosome activated in a mosaic expression pattern. This expression provides the opportunity of developing isogenic lines, in which the X-chromosome carrying the mutation is activated in the affected lines and the healthy chromosome is active in the control lines. However, this approach is challenged, as previous studies have obtained mixed lines due to X-chromosomal reactivation (XCR) after reprogramming with lentiviruses (Barakat *et al.,* 2015). Reprogramming produces iPSC products that either show conserved X-chromosomal inactivation (XCI) or XCR followed by a mosaic or a skewed XCI, indicating that XCI stability during iPSC generation is inconsistent (Marchetto *et al.,* 2015; Pomp *et al.,* 2011). Different reprogramming methods could be the source of different outcomes in the inactivated X-chromosome (Xi) (Cheung *et al.,* 2011; Cheung *et al.,* 2012; Kim *et al.,* 2011, Ananiev *et al.,* 2011). Therefore, identification of a reprogramming method that retains the XCI could lead to the generation of unmixed lines. Unfortunately, depending on the area from where the sample was taken, skin biopsies of patients may contain both affected and healthy fibroblasts in different rates. Furthermore, it has been shown that culturing and maintenance of fibroblasts can lead to X-chromosomal skewing, which will have an additional effect on the cell ratio (Pomp *et al.,* 2011). This will lead to complications when attempting to generate unmixed isogenic lines, especially when ratios are heavily skewed. In the current study we offer a solution to overcome skewness of fibroblast lines and XCR by using a reprogramming protocol that consists of fibroblast pre-sorting and a reprogramming method that does not change Xi.

The aim of the current study was to develop an efficient method to obtain isogenic iPSC lines for X-chromosomal disorders. We focus on Rett Syndrome (RTT), a neurodevelopmental disorder that is often associated with mutations in the X-chromosomal Methyl CpG Binding Protein 2 gene (MECP2). These mutations mainly affect females who show a mosaic expression of mutated MECP2. Here we compared a classic lentiviral reprogramming method (Warlich *et al.,* 2011) with an episomal version (Okita *et al.,* 2011), on mixed and pre-sorted RTT patient fibroblast lines. We show that the iPSCs generated via episomal reprogramming (EiPSCs) did not show XCR of the initial Xi, allowing selection of pure isogenic lines from pre-sorted fibroblasts for functional investigation. On the other hand, iPSCs generated with lentiviruses (ViPSCs) showed a mixed population of MeCP2 expressing cells, even when donor lines all showed XCI of same X-chromosome, thereby hampering the production of pure lines. Based on these results, fibroblast pre-sorting combined with episomal reprogramming appears capable of generating isogenic disease and healthy control lines for iPSC-based studies of X-linked disorders. This could reduce variability and therefore aid discovery of new disease mechanisms and specific targets for therapy.

RESULTS

X-Chromosomal State after Using Different Reprogramming Methods

To find a reprogramming method in which the XCI of the generated iPSCs can be controlled, we created iPSC lines from three Rett patients with MeCP2 deficiencies using both, the classic lentiviral and the non-integrative episomal reprogramming method. We reprogrammed skin fibroblasts from a Rett patient carrying the R255X mutation (RTT-FB R255X), whose fibroblast line was mainly negative for MeCP2 expression (p5%:n95%). After the lentiviral reprogramming, we selected 9 colonies for further passage and characterization (Fig. S1). All lentiviral generated iPSC lines (ViPSCs R255X) showed pure MeCP2-negative colonies in P1–2 (Fig. 1a). After P3, MeCP2-positive cells appeared in these initially negative iPSC lines (Fig. 1b). Beyond passages 4–9, 5 out of 9 iPSC lines consisted of mixed populations of MeCP2-positive and –negative cells (Fig. 1b).

After episomal reprogramming of RTT-FB R255X, we selected and characterized 10 colonies. In contrast to lentiviral reprogramming, colonies generated via episomal reprogramming (EiPSCs R255X) were all MeCP2-negative at every characterized passage (Fig. 1c). To further confirm that episomal reprogramming can generate iPSC populations and maintain the Xi present at the fibroblast stage, we performed the same reprogramming procedures on two more Rett patient fibroblast lines. We episomally reprogrammed fibroblast lines in which the majority of cells were MeCP2-positive (RTT-FB R270X, p97%:n3% and RETT-FB DEL, p70%:n30%). After episomal reprogramming, all 10 iPSC lines from RTT-FB R270X (EiPSC

R270X) and all 10 iPSC lines from RETT-FB DEL (EiPSC DEL) resulted in solely MeCP2-positive iPSC colonies (Fig. 1d+e). All cells in these colonies for both patients remained positive for MeCP2 expression after more than 10 passages (Fig. 1d+e).

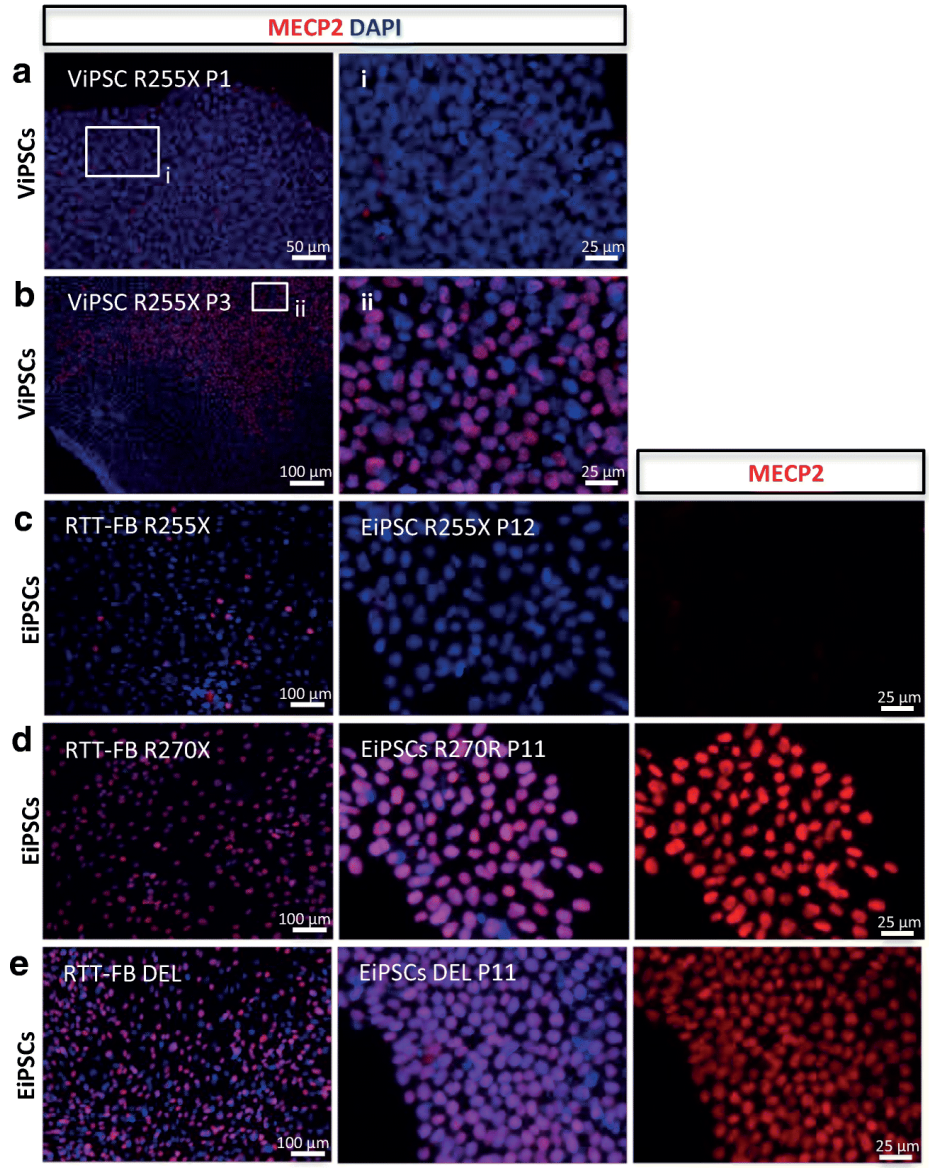


Figure 1: MeCP2 expression after reprogramming. Immunocytochemistry for MeCP2 of **a** patient R255X line after viral reprogramming at ViPSC passage 1 (P1) and **b** passage 3 (P3); **c** patient RTT-FB R255X **d** patient RTT-FB R270X lines and **e** patient RTT-FB DEL at fibroblast stage and after episomal reprogramming (the EiPSC stage), respectively, at passage 12 and 11 (P12+P11)

Single Fibroblast Sorting to Generate Mutated Cell Lines and Isogenic Controls

Selection of specific cell populations from mixed iPSC lines is still challenging. While episomal reprogramming methods can potentially generate an iPSC line with the same Xi, fibroblast lines with low percentages of cells containing the X-linked mutation or the X-linked healthy genetic variant might not generate iPSC lines of both identities (Fig. 1c-e). To overcome these issues, we investigated whether pre-sorting of fibroblasts in combination with episomal reprogramming, would generate pure iPSC lines with the identical Xi.

To create a fibroblast line from RTT-FB DEL with a homogeneous maternal or paternal X-chromosomal state, we plated single cells into a 96-well plate. About 30% of these cells attached and could be passaged further. Fibroblast clones were characterized as pure MeCP2-positive (RTT-FB DEL CTR, Fig. 2a top), pure MeCP2-negative (RTT-FB DEL MUT, Fig. 2a bottom) or mixed population (not shown), using immunocytochemistry and PCR analysis (Fig. 2c). Episomal reprogramming was performed with RTT-FB DEL CTR and RTT-FB DEL MUT fibroblast lines to investigate if their Xi state could be maintained.

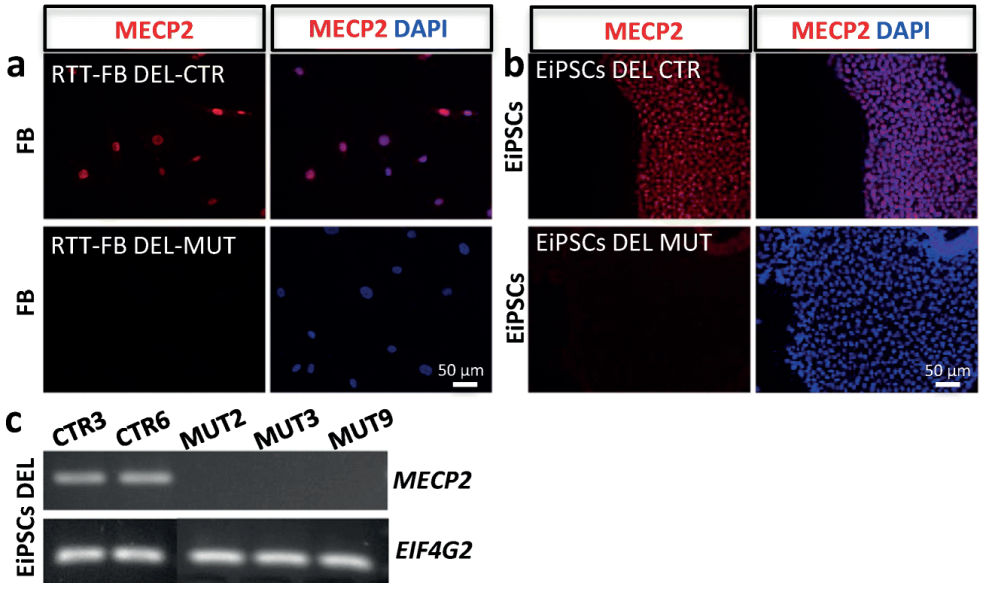


Figure 2: Generation of RTT and isogenic control cell line. Immunocytochemistry for MeCP2 of (**a**) manually-sorted RTT patient fibroblasts. Into MeCP2-positive (upper panels) and –negative (lower panels) cells, and (**b**) the EiPSC-derived lines. **c** PCR analysis of MeCP2 expression of cell lines EiPSC DEL CTR and EiPSC DEL MUT

We picked 10 colonies per fibroblast line and characterized these for MeCP2 expression. PCR analysis confirmed that all picked colonies for the MUT fibroblast line showed a pure population of MeCP2-negative cells up to P14 (Fig. 2c), which is in accordance with the MeCP2 state of the reprogrammed fibroblast line. Furthermore, all iPSC lines derived from the CTR fibroblast line showed a clear positive result for MeCP2 in PCR analysis (Fig. 2c). While PCR analysis does not exclude the presence of MeCP2-negative cells, immunocytochemical inspection for MeCP2 of selected samples showed pure populations of MeCP2-positive cells (Fig. 2b). Therefore, we were able to generate exclusively MeCP2-positive EiPSC colonies from RTT-FB DEL CTR and entirely MeCP2-negative colonies from RTT-FB DEL MUT (Fig. 2b).

Chromatin State during Early iPSC Passages

In previous studies, the X-chromosomal state of cells was investigated by different approaches. Immunocytochemistry for H3K27me3 provides one option for visualizing Xi. An acquisition of this histone methylation is known to support the XCI and can be observed as a high conjugated spot in the nucleus (Rouguelle *et al.,* 2004; Boggs *et al.,* 2002; Plath *et al.,* 2003). To confirm that the viral and episomal reprogramming methods result in different X-chromosomal states in obtained iPSC lines, we characterized the condensation of H3K27me3 by immunostaining. H3K27me3 staining analysis was performed at different passages in two different clones of EiPSC R270X (EiPSC R270X_1 and EiPSC R270X_2) and ViPSC R270X (ViPSC R270X_1 and ViPSC R270X_2). The iPSCs from both reprogramming methods showed a condensed spot of H3K27me3 labeling during the first passages (P1–4) (Fig. 3a and b), which changed after higher passage numbers. At P7, single groups of cell nuclei negative for H3K27me3 appeared in ViPSC clones (Fig. 3b) and increased in cell numbers during further passages. At P10, ViPSC colonies were partly or totally negative for H3K27me3 (Fig. 3b) and stayed mixed up to P15 (Fig. 3b). In contrast, all generated EiPSC lines, partly showing scattered H3K27me3 condensation in some nuclei (Fig. 3a), were positive for H3K27me3 at all passages up to P15 (Fig. 3a).

Furthermore, expression of X-inactive specific transcript (XIST) plays a key role in X-chromosomal inactivation (Duret *et al.,* 2006; Clerc & Avner, 1998; Morey *et al.,* 2004). The expression of XIST in ViPSCs and EiPSCs at P4 and P10 was studied using RNA-Seq analysis (Fig. 3c). At P4, both types of iPSCs show a relatively high expression of XIST RNA. When directly compared no significant difference in XIST expression levels between ViPSCs and EiPSCs was observed (n.s., two-sided t-test), further confirming the presence of XCI at P4 using either methods. However, at P10 ViPSCs showed significantly decreased XIST expression, when compared expression levels of EiPSCs (p value = 0.04, two-sided t-test), indicating XCR in ViPSCs. In contrast EiPSCs showed a stable XIST expression throughout passages (Fig. 3c). To confirm the stability of the XCI in EiPSCs during long term culture, we

have differentiated EiPSCs into neuron according to the protocol described by Nadadhur et al. (2017) (Fig. S2). All neurons show that the XCI of the original fibroblast lines was retained until a mature neural stage. Furthermore, this confirms the potency of the EiPSCs to differentiate into mature neurons despite the lack of XCR.

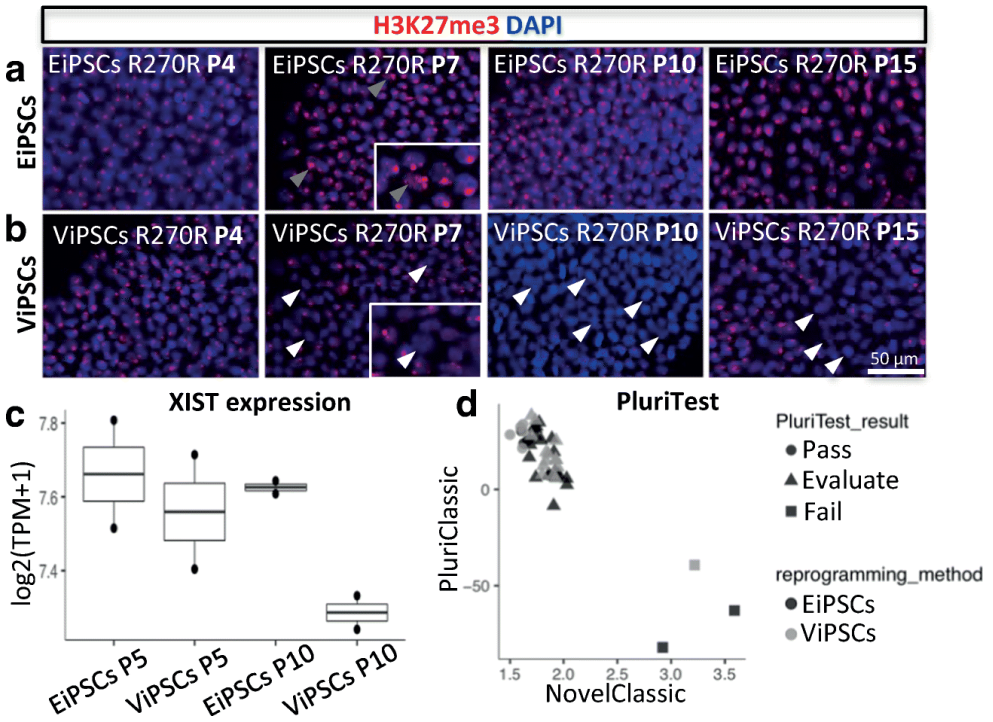


Figure 3: H3K27me3 condensation and XIST expression in early ViPSC and EiPSC passage lines. Immunocytochemistry for H3K27me3 of control iPSC lines at passages P4, P7, P10, P15 generated via **a** episomal reprogramming and **b** viral reprogramming. **c** RNA-Seq analysis of XIST for episomal and viral reprogrammed lines at passage P5 and P10 (two-sided t-test, $n=2$, $p=0{,}04$, data is represented at mean). PluriTest scores for all EiPSCs and ViPSCs

Pluripotent State of iPSCs after Different Reprogramming Methods

To investigate whether the reprogramming methods differently affected the pluripotency state of the iPSC lines, we performed RNA-Seq followed by Pluri-Test analysis (Müller *et al.,* 2011). ViPSCs and EiPSCs equally passed the PluriTest (ViPSCs passed:19,2% evaluate:80,8% EiPSCs passed:17,6% evaluate: 82,4%) and did not cluster separately (Fig. 3d). Pluripotency of iPSC lines was further evaluated by germ layer formation, which showed expression of mesodermal, endodermal and ectodermal markers in the iPSC derived from both methods (Fig. 4). This indicates that both ViPSCs and EiPSCs reached the pluripotency state making them suitable for follow-up studies.

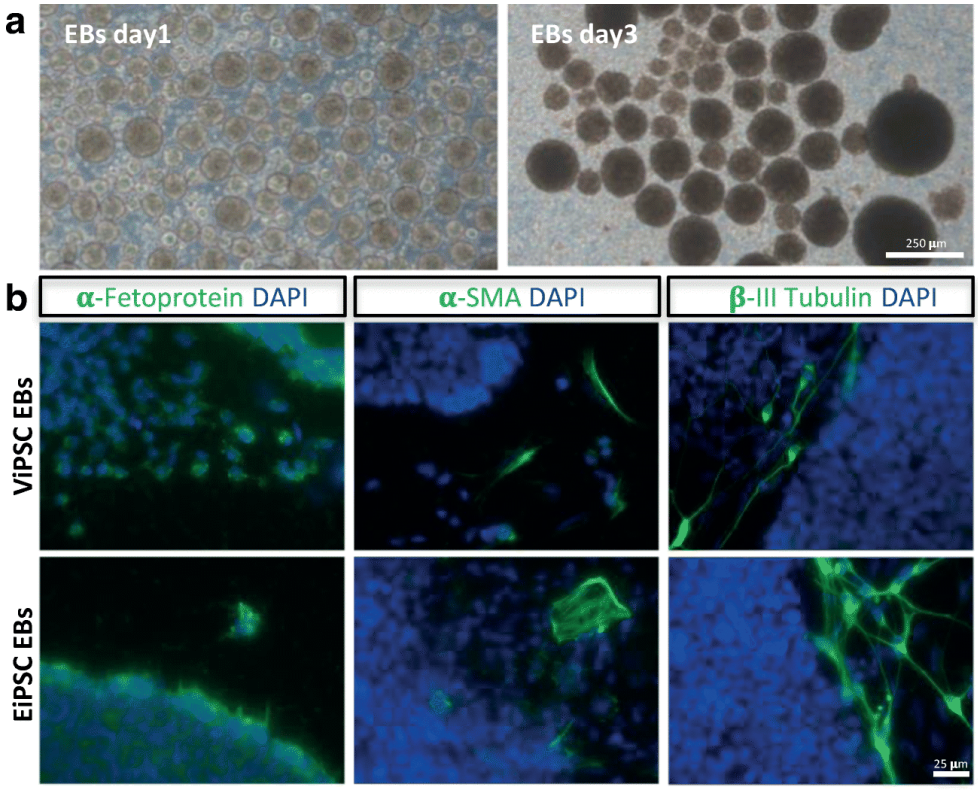


Figure 4: Embryoid Body formation. **a** Brightfield images of day 1 and 3 of embryoid bodies formed from iPSCs. **b** Immunocytochemistry of both ViPSC- derived EBs (upper panels) and EiPSC- derived EBs (lower panels) for all three germ layers: (i) α-Fetoprotein (endoderm), (ii) α-SMA (mesoderm) and (iii) β-III Tubulin (ectoderm)

DISCUSSION

Here we showed that episomal and lentiviral reprogramming affects XCI differently. This observation could aid the generation of iPSC lines from patients with an X-chromosomal disorder with the uniform inactivation of chromosomes carrying either the mutated or healthy gene (i.e. isogenic controls). Based on X-linked expression of MeCP2, we showed that XCI at the fibroblast stage remains stable during episomal reprogramming, but becomes unpredictable during lentiviral reprogramming. Furthermore, based on histone condensation marker H3K27me3 and XIST expression we showed XCR in a subset of cells generated via viral reprogramming method. This is in accordance with the mixed population of MeCP2-positive and –negative cells seen in colonies of ViPSC R255X, which appeared from P3 onwards (Fig. 1b). In contrast, all passages of EiPSCs showed stable MeCP2-expression

consistent with their donor fibroblasts (Fig. 1c-e). The stable MeCP2 expression shown in EiPSCs, is an indication that episomal reprogramming in pre-sorted fibroblast lines can be a reliable, efficient and less cost-intensive method to generate affected iPSCs and isogenic controls for the investigation of X-chromosomal-linked disorders, such as Rett syndrome.

Robust Generation of Isogenic Controls by Episomal Reprogramming

Lentiviral reprogramming of the mostly MeCP2-negative fibroblast line RTT-FB R255X led to XCR, resulting in a mixed population of MeCP2-positive and –negative iPSCs. However, this was not the case for every ViPSC R255X clone. Four out of nine lines stayed negative for MeCP2, while the remaining five lines showed different ratios of MeCP2-positive and -negative cells. Even though purely negative lines were generated by lentiviral reprogramming, none of our lines was entirely positive for MeCP2. Therefore, as described before (Barakat *et al.,* 2015; Cheung *et al.,* 2011; Ananiev *et al.,* 2011), lentiviral reprogramming is unpredictable and can lead to a random XCI after reprogramming and during iPSC maintenance.

In contrast, episomal reprogramming of mainly MeCP2-negative fibroblast line RTT-FB R255X only generated EiPSC lines with MeCP2-negative cells, which did not show MeCP2 expression throughout all analysed passages (Fig. 1c). This suggests that episomal reprogramming does not reactivate the silenced X-chromosome. We confirmed this by the episomal reprogramming of the mainly MeCP2-positive fibroblast line RTT-FB DEL and RTT-FB R270X. All iPSC lines derived from the latter fibroblasts were MeCP2-positive and maintained their MeCP2 identity for over 12 passages (Fig. 1d+e). Nevertheless, XCR could have taken place, as the affected cells would still have shown a MeCP2 positive staining. However, RNA sequencing showed stable levels of XIST expression throughout several passages, suggesting no XCR took place in these cells (Fig. 1c). This implies a stable Xi within the EiPSCs after episomal reprogramming, which depends on the XCI of the used fibroblasts. This was further confirmed by long-term differentiation towards neurons, where all cells retained the original MeCP2 expression of the fibroblast line they were derived from (Fig. S2).

To generate purely MeCP2- positive and –negative iPSC lines from a mixed fibroblast line, single-cell-plated fibroblasts were expanded and characterized for their MeCP2-state. The cloned fibroblast lines were used to generate pure MeCP2-mutant and isogenic control lines of the same patient. Previous studies had to perform several rounds of reprogramming in order to generate isogenic iPSC lines due to skewing issues (Pomp *et al.,* 201; Cheung *et al.,* 2011; Ananiev *et al.,* 2011). Therefore, our approach involving single-cell-plating fibroblasts and opting for episomal reprogramming, can be a more efficient and less cost-intensive method.

Pluripotency Presents with Different X-Chromosomal States

In vivo, the naïve pluripotent stem cell state in female cells is referred to a state in which both X-chromosomes are active (Gafni *et al.,* 2013). However, earlier studies indicated that in vitro, XCI is induced by passaging, maintenance and early differentiation of pluripotent stem cells into somatic cells (Lengner *et al.,* 2010). While all of our lines show the standardized pluripotency markers (Fig. S1), this could suggest that ViPSCs are more naïve than EiPSCs. This could not be confirmed by the characterisation of EiPSC and ViPSCs using RNA seq. The results of the Pluri-Test showed no separate clustering of the different iPSCs. Furthermore, Pluri-Test in combination with classic characterization analysis indicated that both ViPSC and EiPSC lines are pluripotent.

iPSC Reprogramming Induces Poised XCI

The absence of H3K27me3 shows XCR in the lentiviral reprogramming method. As we observed disappearance of H3K27me3 in nuclei of the ViPSCs from P7 onwards but not in EiPSCs, we conclude that the XCR only establishes beyond early passaging. This difference became more obvious after P10, in which we observed big parts or entire colonies of ViPSCs to be negative for H3K27me3. To ensure XCR was not delayed but lost in EiPSCs we cultured both lines up to P15. No H3K27me3 negative nuclei appeared in EiPSCs (Fig. 3a), while we clearly observed them in ViPSCs (Fig. 3b). Although EiPSCs were positive for H3K27me3 at all passages, clear chromosomal condensation was sometimes partly lost, thereby creating a more scattered signal (Fig. 3a). A possible explanation for this is that episomal reprogramming led to partial reactivation of both X-chromosomes, but due to epigenetic memory and skewing, the formerly inactivated chromosome is again inactivated (Pomp *et al*., 2011). However, this seems implausible since we did not observe MeCP2 expression at any stage during episomal reprogramming in line EiPSC-R255X, suggesting that the X-chromosome was inactive throughout cultivation. Another possible explanation is, that even though the chromosome seems to be partly unwound, it remains inactive due to the expression of other factors involved in Xi, such as XIST (Petruk *et al.,* 2017). RNA-Seq revealed a decreased XIST expression at P10 in ViPSCs, which coincides with the loss of H3K27me3 condensation. The same effect was not observed in EiPSCs, as XIST expression remained stable. These differences in XIST expression might explain why viral reprogramming leads to reactivation of the silenced X-chromosome, while episomal reprogramming does not (Pomp *et al.,* 2011; Cheung *et al.,* 2012; Sahakyan *et al.,* 2017). Taken together, the absence of H3K27me3 condensation in ViPSCs and decreased XIST expression suggests XCR. As no changes in expression of XIST or H3K27me3 were seen in EiPSCs, in combination with uniform MeCP2 stainings, we conclude episomal reprogramming does not affect the X-chromosomal state of donor cells.

Proper Controls in iPSC-Based Models for X-Linked Disorders

There is currently more awareness that genetic background could contribute to disease phenotypes in iPSC-based studies and should be accounted for (Hoekstra *et al.,* 2017). Especially when genes of interest, such as MeCP2, interact with other genes and different pathways, the generation of isogenic controls can be very important. Absence of isogenic controls weakens conclusions regarding causal effect of the mutation alone on observed phenotype. Our approach to generate proper controls, is not only useful for development of in vitro models of RTT, but also for other dominant X-linked diseases like Fragile X or Coffin-Lowry syndrome (Amir *et al.,* 1999; Rocchi *et al.,* 1990; Fryssira *et al.,* 2002).

Taken together, the presented method of generating disease and control lines from patients with dominant X-chromosomal disorders facilitates in vitro iPSC-based disease modelling, which can be hindered by high variability. It offers an efficient and inexpensive approach to generate iPSC populations with either only the mutated or the healthy X-chromosomes. This approach, in combination with other recent advances in the stem cell field to reduce variability, such as standardization and automation, are of utmost importance to generate valid disease models.

MATERIALS AND METHODS

The investigation of differences in XCR after reprogramming was performed on Rett Syndrome patient cells. We reprogrammed RTT patient fibroblasts via two different reprogramming methods and compared the generated iPSCs. This comparison was done by several assays as immunocytochemistry and RNA-Seq. The generated iPSCs were then differentiated towards all three germ layers via Embryoid Body formation to confirm their pluripotency. Furthermore, neurons were generated from iPSC lines to investigate XCI. All experiments were exempt from approval of Medical Ethical Toetsingscommissie (METC), Institutional Review Board of the VU medical centre.

Cell Culture

Fibroblasts from three female Rett syndrome patients were obtained from the *Cell lines and DNA bank of Rett Syndrome, X-linked mental retardation and other genetic disease*, member of the Telethon Network of Genetic Biobanks. Each fibroblast line carried a different mutation: Deletion within Exon 3 and 4 of *MECP2*, nonsense mutation p.R255X and p.R270X mutation (here referred to as RTT-FB DEL, RTT-FB R255X and RTT-FB R270X).

Cells were expanded in Fibroblast medium (DMEM-F12, 20% FBS, 1%NEAA, 1%Pen/Strep, 50 μM β-Mercaptoethanol). To generate pure Rett fibroblast lines (RETT-FB DEL MUT) and isogenic controls (RETT-FB DEL CTR) cells were dissociated and single cells were seeded

in 96-well plates. After expansion, fibroblasts were tested for MeCP2 expression by PCR and immunocytochemistry.

Reprogramming

The viral reprogramming was performed using a lentiviral construct containing the classical reprogramming factors *OCT4*, *SOX2*, *KLF4* and *C-MYC* (Warlich *et al.,* 2011).

Episomal reprogramming was achieved as described before with small adjustments (Okita *et al.,* 2011). Fibroblasts were dissociated from cell culture plates with Trypsin-EDTA (0,05%) (ThermoFisher). To prepare one well of a 6-well plate, 4×10^5 cells were collected and centrifuged for 5 min at 1200 rpm. Pellet was washed once with PBS and re-suspended in 400 µl Gene Pulser® Electroporation Buffer Reagent (BioRad) containing 23.3 µg of each episomal plasmid (Addgene, Plasmid #27078, #27080, #27076). Cell-plasmid suspension was electroporated in electroporation cuvettes with Gene Pulser II (BioRad). Three pulses of 1.6 kV, with a capacitance of 3 µF and a resistance of 400 Ω were applied. Cells were left to recover overnight in Fibroblast medium without antibiotics but with 10 µM ROCK-inhibitor (Y-27632) in Geltrex®-coated well of a 6 well plate. The next day, medium was changed to Fibroblast medium with antibiotics.

When reprogrammed fibroblasts reached 60–70% confluence, medium was changed to TeSR™-E7™ (STEMCELL) and refreshed daily. Colonies were picked manually after 21–28 days and maintained in TeSR™-E8™ (STEMCELL) on Vitronectin XF™ (STEMCELL) coated 6 well plates (Holmes & Heine, 2017).

Embryoid Body Formation and Neuronal Differentiation

To generate Embryoid Bodies (EBs) and test the ability of iPSC lines to form all three germ layers, one well of a 6-well plate of EiPSCs and ViPSCs were dissociated from Vitronectin XF™ (STEMCELL) coated plates with Gentle Cell Dissociation Reagent (STEMCELL). After 5 min incubation Gentle Cell Dissociation Reagent was aspirated and TeSR™-E8™ with 10 µM ROCK-inhibitor was added. Cells were detached by tapping them off the plate and transferred into an anti-adhesive Poly(2-hydroxyethyl methacrylate) (Sigma-Aldrich) coated well of a 6-well plate. Next day, EB-formation was checked and half of the medium (TeSR™-E8™ with 10 µM ROCK-inhibitor) was changed. Floating EBs were cultured for 10 days and half of medium was replaced every other day with TeSR™-E8™. After 10 days EBs were plated on Geltrex®-coated coverslips in TeSR™-E8™ and kept in culture for another 3 days. Afterwards, EBs were fixated and stained for germ layer markers. Neuronal differentiation was performed as described before (Nadadhur *et al.,* 2017). Generated neurons were fixated and immunocytochemically stained for neuronal marker and MeCP2.

Immunocytochemistry

For immunocytochemistry, cells were plated on Vitronectin XF™-coated coverslips and fixated with 4% paraformaldehyde for 10 min followed by PBS wash (3×). Blocking and permeabilization were performed by a one-hour incubation in blocking buffer (PBS, 5% normal goat serum (Gibco®), 0.1% bovine serum albumin (Sigma-Aldrich), 0.3% Triton X-100 (Sigma-Aldrich)). Primary antibodies for MeCP2 (D4F3, CellSignaling, 1:200, rabbit), H3K27me3 (C36B11, CellSignaling, 1:1600, rabbit), OCT4 (C-10, Santa Cruz, 1:1000, mouse), SSEA4 (Developmental Studies Hybridoma Bank, 1:50, mouse), TRA1–60 (Santa Cruz, 1:200, mouse), TRA1–81 (Millipore, 1:250, mouse), SOX2 (Millipore, 1:1000, rabbit), β-III Tubulin (R&D Systems, 1:1000, mouse), α-Fetoprotein (R&D Systems, 1:1000, mouse), α-Smooth Muscle Actin (SMA) (Progene, 1:1000, mouse), MAP2 (Abcam, 1:500, chicken), SMI312 (Eurogentec, 1:1000, mouse) and VGLUT2 (SYnaptic SYstems, 1:1000, rabbit) were diluted in blocking buffer and incubated for 1 h at room temperature followed by an overnight incubation at 4 °C. After PBS wash, secondary antibodies Alexa Fluor® 488 (ThermoFisher, 1:1000, mouse) and Alexa Fluor® 594 (ThermoFisher, 1:1000, mouse or rabbit) diluted in blocking buffer were applied to the cells for 1–2 h at room temperature. DAPI staining was performed in PBS for 5 min and coverslips were mounted on glass slides with Fluoromount™ (Sigma-Aldrich).

RT-PCR

Total RNA from EiPSCs and ViPSCs was collected after ten passages (P10) and isolated by standard TRIzol®-chloroform isolation and iso-propanol precipitation. cDNA was derived from the collected mRNA by using SuperScript IV Reverse Transcriptase (SSIV) (ThermoFisher). For RT-PCR 1 µg of RNA and Random Hexamer Primers were used and incubated with SSIV, 0,1 M Dithiothreitol (DTT) and 40 U/µl RNaseOUT (ThermoFisher) for 10 min at 23 °C, 10 min at 50 °C and 10 min at 80 °C. Afterwards samples were stored at −20 °C until further processed. PCR analysis was performed with different primer sets (Tab. S1) with Phire Hot Start II DNA Polymerase (ThermoFisher). To perform PCR, a mastermix containing Phire Buffer (ThermoFisher), dNTPs (10 mM) (ThermoFisher) and Phire Hot Start II DNA Polymerase was mixed and divided over different PCR tubes. 1 µl of each primer (10 µM) and 1 µl cDNA was added to the tubes and carefully mixed. Samples were placed in thermocycler (Applied Biosystems™ 2720 Thermal Cycler) and PCR program was initiated (Tab. S2).

RNA Sequencing

Total RNA was isolated from iPSCs during passaging using TRIzol®-chloroform isolation as described above. RNA quality was evaluated by calculating the RNA integrity number (RIN; Agilent Technologies) using an Agilent 2200 TapeStation system (tape D5000). Samples with RIN ≥ 8 were selected and used to prepare libraries for RNA sequencing using the TruSeq

mRNA sequencing kit (Illumina) according to manufacturer's recommendations. Loading concentration of 100 to 200 ng RNA were used for the library preparation. Briefly, mRNA was purified using poly-T oligo magnetic beads and then fragmented. Reverse transcription was performed using SuperScript IV Reverse Transcriptase (SSIV) (ThermoFisher) and First Strand Synthesis Act D Mix (Illumina) followed by production of the second strand with incorporation of dUTP using Second Strand Marking Master Mix (Illumina) and purification of samples. This was followed by A-tailing and adaptor ligation (Ligation Mix and Stop Ligation Buffer, Illumina). Samples were washed using the AMPure XP Beads (Beckman Coulter) DNA fragments were enriched using PCR Primer Cocktail and PCR Master Mix for 15 cycles (Illumina) and a last purifications step was performed. Library quality control was performed using the Agilent Technologies 2100 Bioanalyzer where after samples were sequenced with the Illumina HiSeq 4000 (50 base pair single read).

Quality control was performed using FastQC software and sequencing reads aligned to Human Genome hg38 by STAR v2.5.3a (Dobin *et al.,* 2013). Expression value was quantified as, expected count and transcripts per million (TPM), using RSEM v1.3.0 (Li & Dewey, 2011) with GENCODE v25 gene annotation (http://www.gencodegenes.org/) which contains 63,299 genes.

PluriTest

PluriTest was performed for samples by submitting fastq file to the PluriTest server (https://pluritest.org/) (Müller *et al.,* 2011).

Statistics

A T-Test was performed to compare the expression levels of *XIST* between EiPSCs and ViPSCs using R programming language software.

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde

R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380.e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J,

White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity. Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal

dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684. https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of “Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox.” (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of

GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of

Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B, Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of

induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

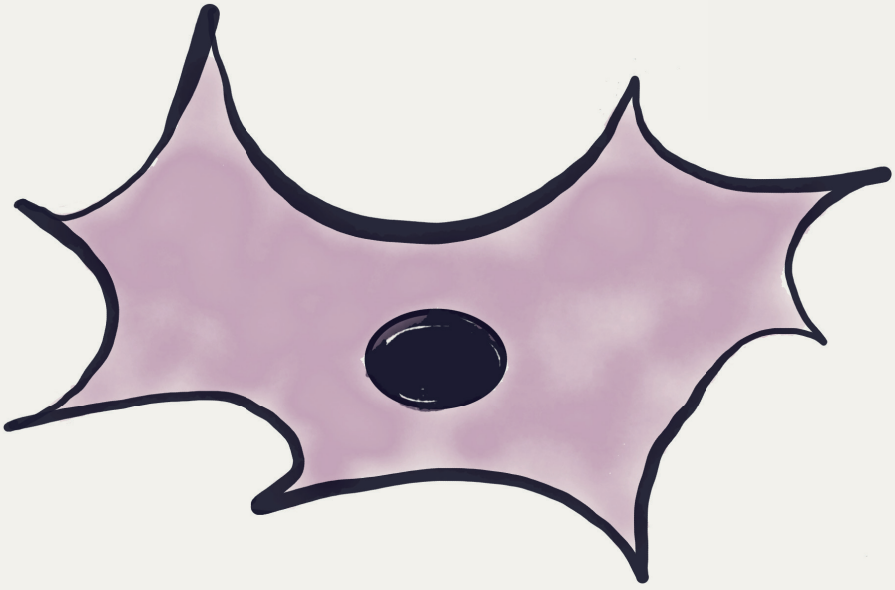
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Chapter 4

Systematic assessment of variability in the proteome of iPSC derivatives

Stephanie D. Beekhuis-Hoekstra[a], Kyoko Watanabe[a] , Josefin Werme[a] , Christiaan A.de Leeuw[a] , Iryna Paliukhovich[b] , Ka Wan Li[b] , Frank Koopmans[b] , August B. Smit[b] , Danielle Posthuma[ac] & Vivi M.Heine[ac]

[1]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands.
[2]Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands.
[3]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands; Department of Child and Youth Psychiatry, Emma Children's Hospital, Amsterdam Neuroscience, Amsterdam UMC, Amsterdam, The Netherlands. Electronic
[4]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands; Department of Child and Youth Psychiatry, Emma Children's Hospital, Amsterdam Neuroscience, Amsterdam UMC, Amsterdam, The Netherlands. Electronic

Published online October 2021 in Stem Cell research Volume 56, October 2021, 102512

ABSTRACT

The use of induced pluripotent stem cells (iPSC) to model human complex diseases is gaining popularity as it allows investigation of human cells that are otherwise sparsely available. However, due to its laborious and cost intensive nature, iPSC research is often plagued by limited sample size and putative large variability between clones, decreasing statistical power for detecting experimental effects. Here, we investigate the source and magnitude of variability in the proteome of parallel differentiated astrocytes using mass spectrometry. We compare three possible sources of variability: inter-donor variability, inter- and intra-clonal variability, at different stages of maturation. We show that the interclonal variability is significantly smaller than the inter-donor variability, and that including more donors has a much larger influence on statistical power than adding more clones per donor. Our results provide insight into the sources of variability at protein level between iPSC samples derived in parallel and will aid in optimizing iPSC studies.

INTRODUCTION

Human induced pluripotent stem cell (iPSCs) technology (Takahashi and Yamanaka, 2006) has provided new opportunities for studying human- and disease-specific cellular mechanisms. This has been especially beneficial to brain-related disorders, for which living human cells and tissue are often not available for experimental study. As the genetic make-up of neuronal cells derived from iPSCs is identical to the donor, the use of iPSC-derived neuronal cells as a model for genetically complex disorders does not require a-priori knowledge of the actual causal genes, and is not limited to monogenic diseases. Therefore, the use of iPSCs is becoming of particular interest for genetically complex disorders such as schizophrenia and autism spectrum disorders (Hoekstra et al., 2017). Experimental iPSC studies are however laborious and cost intensive, and often involve a limited number of cell lines (in the order of 2–14 lines). Any factor that creates variation in targeted experimental effects will lower the statistical power to detect the elicited experimental effects. Since the number of cell lines used is generally relatively low, a study design that optimally balances the number of donors and the number of generated clones per donor is needed. Previous studies have reported on the presence of transcriptional variability between and within iPSC lines (Vitale, 2012, Kyttälä et al., 2016, Carcamo-Orive et al., 2017, Rouhani, 2014, Schwartzentruber, 2018). To the best of our knowledge we are the first to report on variability of differentiated iPSC-derivatives at protein level.

Variability in iPSCs can be categorized in three types (i) interdonor variability: differences between samples from different donors, (ii) interclonal variability: differences between clones from the same donor, and (iii) intraclonal variability: differences between batches from the same clone. The first category, interdonor variability, is expected to be the greatest source of variability due to genetic differences, which is confirmed by Rhouhani et al (2014) and Kyttala et al (2016). In case of a genetic underpinning of disease, interdonor variability also manifests the biologically relevant effect. Although interclonal variability is expected to be lower than variability between donors (since clones are genetically identical), this has actually been shown to vary (Vitale, 2012, Kyttälä et al., 2016, Rouhani, 2014, Schwartzentruber, 2018). Interclonal variability can arise (at least in part) from genetic mutations occurring during culturing which lead to (small) genetic variation between clones (Laurent et al., 2011). A second potential source of interclonal variability is alterations in DNA methylation, which can remain from the original somatic cell or can arise throughout culture and may depend on the age and sex of the donor and the reprogramming method used to derive the iPSCs (Salomonis et al., 2016, Fossati et al., 2016, Nishizawa et al., 2016, Bock et al., 2011). Intraclonal variability can arise from variation in culturing conditions, affecting the differentiations of a clone in different ways. Schwartsentruber and colleagues (2018) have shown that intraclonal variability in the transcriptome of iPSC-derived sensory neurons is

greater than the variability in primary sensory neurons, and in some cases can be as high as interdonor variability. The latter will strongly affect the power of experiments involving iPSCs in a negative manner and disputes whether iPSCs are suited to model complex disorders.

We here aim to systematically assess what is the most optimal experimental iPSC design in terms of lowering variability and increasing statistical power to detect experimentally induced effects. We further aim to generate practical guidelines for sample inclusion in single-lab iPSC studies, with regard to the number of iPSC lines and clones per donor, to obtain robust experimental results. Whereas previous studies have provided valuable insight into the use of multiple clones and the incorrect use of statistical methods on research sensitivity and spurious findings (Germain and Testa, 2017), we focus on performing the analysis on end products, i.e., in-house iPSC lines, clones and differentiated populations generated in parallel, and on studying protein level changes. We apply an experimental design in which we use samples from three healthy donors, three clones from one each donor, and differentiation into 115-day-old astrocytes. Samples are collected at different stages throughout differentiation and subjected to SNP array analysis and mass spectrometry (MS) in order to investigate variability at the DNA and protein level, respectively, at different time points of maturation (Fig. 1). Based on our experimental data, we conduct simulations to predict the effects of increasing the number of donor and of clones per donor on the power to detect experimental effects or true differences between groups.

RESULTS

Study design

To investigate the level and source of variability in iPSC derivatives, we selected three healthy donors from whom we derived three iPSC clones each which were subsequently differentiated into astrocytes (overview in Fig. 1). Of the three clones per donor, one clone was differentiated three times in parallel into astrocytes to determine the reproducibility of the differentiation procedure into astrocytes (i.e. intraclonal variability). We will refer to these samples as differentiation replicates. This resulted in 9 iPSC lines and 15 iPSC differentiations. Samples were collected at different time points throughout differentiation and were subjected to SNP array and proteomics analysis to investigate variability at different levels. All samples were generated and cultured in-house and in parallel by the same researcher, to avoid interference by other confounding factors, e.g. different handling or inter-lab variability (Volpato et al., 2018). This experiment allows us to analyze variability at different stages (iPSC and astrocyte stage) at different levels (protein and DNA level) and to reveal the source of variability (genetic instability, reprogramming and/or instability of the differentiation process).

Mass spectrometry quality control

To study interdonor, inter- and intraclonal variability in protein levels between donor fibroblasts, iPSCs and iPSC-derived astrocytes, we performed MS analysis using a triple time-of-flight (TOF) MS. The number of proteins detected and their average expression per sample is given in Supplementary Fig. 1a. One sample was measured 8 times (strategically distributed between all other samples; these measurements will be referred to as technical replicates) and the correlation between these technical replicates determined the technical variation of the MS. The median Spearman correlation (ρ) between technical replicates was 0.91, indicating that the technical variability of the MS was in the normal range of Data Dependent Acquisition analysis (Bruderer et al., 2015). Furthermore, iPSC clones were characterized for pluripotency using standardized assays (Supplementary Fig. 1b and Supplementary Fig. 2). Two iPSC samples did not pass QC and were excluded from further analysis.

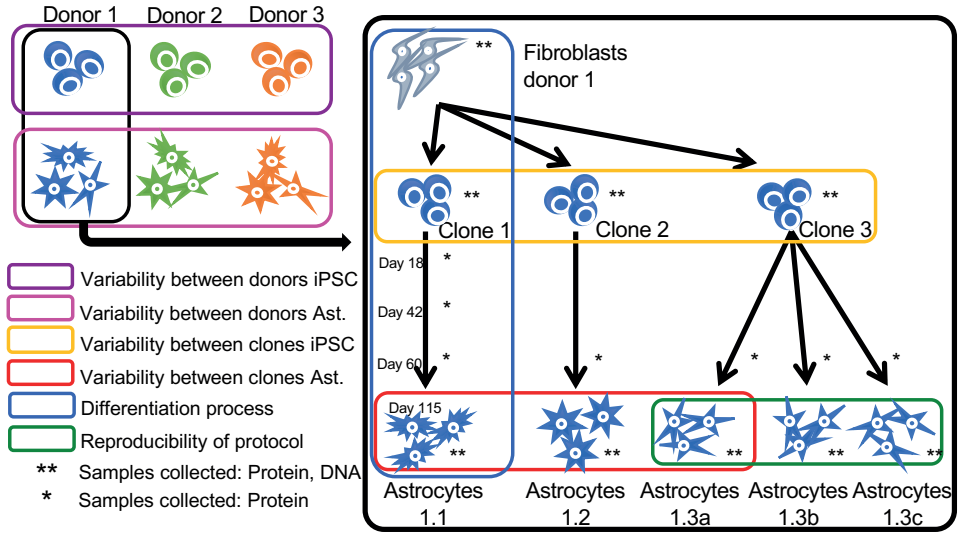


Figure 1: Schematic illustration of experimental set up. Fibroblasts for three individuals were episomally reprogrammed and three iPSC clones per individual were selected. All selected clones were differentiated towards astrocytes with a customized differentiated protocol. One clone per individual was differentiated three times in parallel. Samples were collected throughout the protocol (protein samples (*) or protein and DNA (**). Note that the right panel of the scheme was performed for three different individuals. Please note that the figures and tables displayed in this article are color coded to the rectangles of figure 1 to facilitate the comprehension of the different correlations mentioned in the text and tables.

Validation of iPSCs and differentiated cell types

At days 18, 42, 60 and 115 of differentiation, iPSC derivatives were collected and checked for astrocyte identity by immunocytochemistry (Supplementary Fig. 3) and MS (Supplementary Fig. 1c-e). To further confirm cell stage identity of all samples, we performed an unsupervised hierarchical clustering of samples based on Spearman correlation (ρ) and Wilcoxon Sum of Ranks test (two-sided) on whole proteome data (Clustering is depicted in Fig. 2a and results of Wilcoxon Sum of Ranks test are summarized in Table 1). We found that samples cluster by cell type, i.e. fibroblast, iPSCs (within iPSC samples: median ρ = 0.90; between iPSC and other 2 cell type samples: median ρ = 0.59, $p < 0.01$) and astrocytes (within astrocyte samples: median ρ = 0.76; between astrocyte and other 2 cell type samples: median ρ = 0.69, $p < 0.01$; Fig. 2 and Table 1). The fibroblasts clustered together between the day 60 astrocytes, which could suggest abnormal expression of fibroblast genes as iPSC lines are being differentiated, or this could be due to the visual limitation of a graph with a single dimension. To verify that the astrocytes do not converge back into fibroblasts, we generated a tSNE plot of our samples, which showed separate clustering of the fibroblasts, confirming they are visibly different from astrocytes at any stage (Supplementary Fig. 4). Further analyses showed that samples tend to cluster by differentiation state. Differentiation day 18 samples clustered as a separate group (within day 18 samples: median ρ = 0.88; between day 18 samples and other samples: ρ = 0.73; $p < 0.01$; Fig. 2a and Table 1). The same was observed for differentiation day 115 samples (within day 115 samples: median ρ = 0.81; between day 115 samples and other samples: median ρ = 0.65; $p < 0.05$; Fig. 2a and Table 1). Differentiation day 42 and 60 samples did not cluster as separate groups (Table 1, Fig. 2a). Together, using ICC and MS data, we were able to identify cell type-specific (fibroblast, iPSC, and astrocyte), and early (day 18) and late (day 115) differentiation stage-specific samples.

Table1: Statistical comparison between correlation of groups. Groups are color coded accordingly to Fig. 1.

		Description		p	Median1	Median2
Cell type confirmation		within ipsc	between cell types (excluding astrocytes tech. rep.)	4,33E-14	0,897057	0,591735
		within astrocytes	between cell types (excluding tech. rep.)	1,86E-05	0,756111	0,692084
		within D18	between days	0,000492611	0,883619	0,729645
		within D42	between days	0,225615764	0,810518	0,783419
		within D60	between days	0,845320197	0,754473	0,731492
		within D115	between days	0,026108374	0,814573	0,64965
Correlation analysis	iPSC	within ipsc clone	between ipsc clones	0,008157649	0,931857	0,829741
	Day 60	within differentiationreplicates at D60	between astrocytes at D60 within the same individual	0,631433006	0,878262	0,884301
		within the same sample in D60	between samples in D60	0,011073759	0,884467	0,77962
	Day 115	within technical replicates	between technical replicates and all other astrocytes (D115, 787)	0,067026313	0,914664	0,836865
		within technical replicates	between technical replicates and clones	2,06E-14	0,914664	0,752805
		within technical replicates	between technical replicates and differentiation replicates	2,64E-03	0,914664	0,921307
		within differentiation replicates at D115	between astrocytes at D115 within the same individual	7,69E-04	0,900869	0,780689
		within the same sample in D115	between samples in D115	0,541142055	0,806783	0,809321
	Overall	within astrocytes D115	within ipsc	1,86E-05	0,807744	0,897057
		witin sample D60	within sample D115	0,161497326	0,878262	0,900869
		between sample D60	between sample D115	0,006615716	0,764781	0,792931

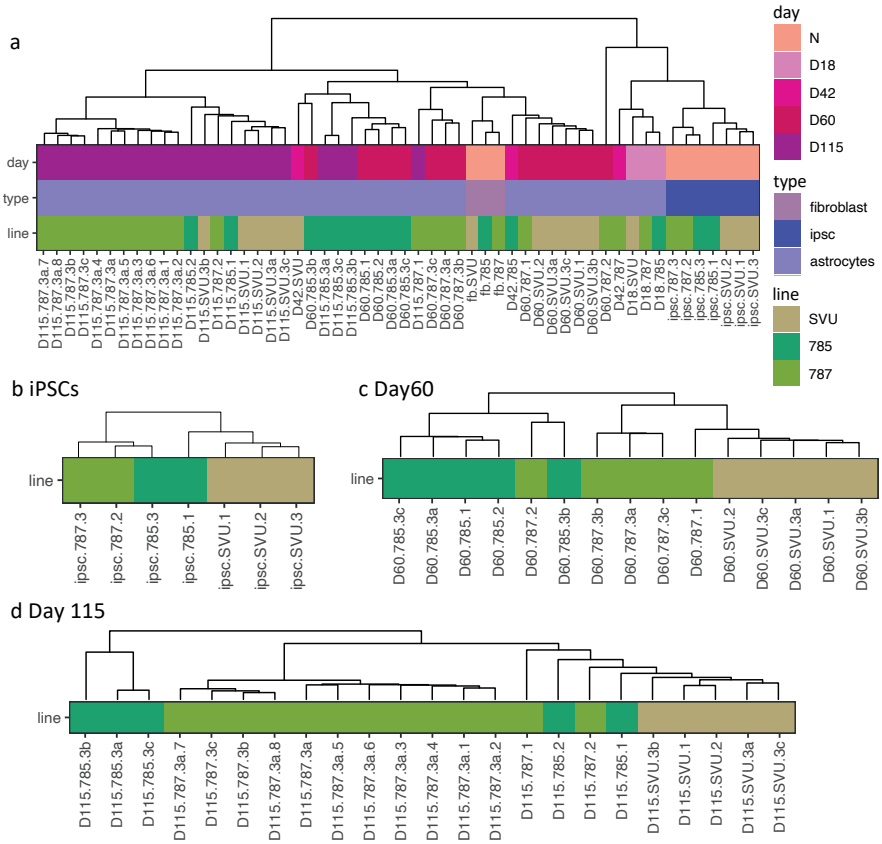


Figure 2: Unsupervised hierarchical clustering of samples based on Spearman Correlation (intensity). Clustering was performed based on the expression of all proteins. **a)** This figure reveals that samples cluster primarily based on cell type, and that within one cell type, samples tend to cluster according to donor. **b)** Hierarchical clustering of iPSCs, **c)** Day 60 samples and **d)** Day 115 samples. For all cell types, clones cluster together according to donor, with a few exceptions.

Sample variability in the proteome between donors and clones

To investigate the variability between donors vs. clones from 1 donor, we evaluated the intersample correlation (ρ). At the iPSC stage (2 or 3 clones per subject), the correlation between clones from the same donor was higher (median $\rho = 0.93$) than iPSCs from different donors ($\rho = 0.83$, $p < 0.01$, Wilcoxon Sum of Ranks Test; Table 1, Fig. 2b). This is also the case at differentiation day 60, where the correlation between clones from the same donor (median $\rho = 0.88$) was higher than between astrocytes from different donors (median $\rho = 0.78$; $p < 0.05$; Table 1, Fig. 2c). Furthermore, the differentiation replicates from the same clone (median $\rho = 0.88$) showed the same correlation as differentiation samples of 3 clones from

the same donor (median ρ = 0.88, n.s.), indicating that the inter- and intraclonal correlation did not vary at differentiation day 60. By differentiation day 115, the higher correlation between clones from one donor compared to astrocytes from different donors was abolished (median ρ = 0.81 for both, n.s.; Table 1, Fig. 2d). Of note, at day 115, within one donor, the differentiation replicates (median ρ = 0.90) were significantly more correlated ($p < 0.01$) than the differentiations from the three different clones (median ρ = 0.78). In summary, up to day 60, the variability between clones from one donor is lower than between astrocytes from different donors, which was abolished by day 115. However, at day 115, differences induced by the differentiation protocol are lower than interclonal differences.

We further explored why the difference in correlation between clones and donors seen at day 60 is abolished by day 115. First, we compared the correlation between all iPSC (median ρ = 0.90) and between all differentiation day 115 astrocyte samples (median ρ = 0.81; $p < 0.01$, Table 1), which showed a reduced correlation over time. This could imply an increase in variability between clones as time progresses. However, no decrease in correlation between clones from one donor (interclonal variability) from day 60 to day 115 was observed (median ρ = 0.88 and median ρ = 0.90 respectively, n.s.), but interestingly, the correlation between different donor samples (interdonor correlation) slightly increased from day 60 to day 115 (ρ = 0.76 at day 60 and ρ = 0.79 at day 115, $p < 0.01$). These results suggest that throughout long-term culturing, the correlation between astrocytes from one single donor remains stable while the astrocytes from different donors become more similar. Therefore, the lack of a significant difference in correlation between clones and donors at day 115 is due to an increase in correlation between donor samples rather than increase in variability between clones.

Sample variability between donors and clones due to copy number variations

A potential cause of the decrease of correlation in the cellular proteome between clones could be the introduction of genetic mutations. To test this, we called copy number variations (CNVs) for iPSCs and for day 115 astrocytes using iPsychCNV package in R (Bertalan et al., 2015). After quality control (Methods), we identified an average of 3.8 CNVs per sample (3.4 deletions and 0.4 duplications, mean deletion size is 994,650 bp, and mean duplication size is 1,4482,786 bp). Interestingly, not all CNVs found at the iPSC stage were found in the astrocytes from the same line. This could either indicate the presence of false positives in our CNV calling, or negative selection against these 'mutant' cells during culturing. None of the *de novo* CNVs spanned genes that were expressed in the measured proteome. Therefore, we were not able to analyze the impact of *de novo* deletions and duplications on the expression level of genes within these regions. We did investigate whether lines carrying large numbers of CNVs clustered separately from its clones based on the proteome profile. We found that the number of CNVs at iPSC stage did not predict clustering (i.e. samples carrying a large

number of CNVs did not necessarily cluster separately from same patient clones), suggesting that CNVs spanning regions that are not expressed by the cell population of interest, do not necessarily increase variability in the proteome.

Exploring variability through coefficient of variance of the proteome

Further exploration of the variability between samples was performed by computing the coefficient of variance (CV) for all proteins. The CV (standard deviation/average) captures the scaled variability of each protein and is expressed as log10(CV) per donor. CVs were compared between several protein groups: a) whole proteome data set (Fig. 3a), b) astrocyte proteins, i.e. upregulated in astrocytes, (Fig. 3b), c) non-cell type associated proteins, i.e. not upregulated in fibroblast, iPSC or astrocytes (Supplementary Fig. 5a), d) fibroblast proteins, i.e. proteins upregulated in fibroblasts (Supplementary Fig. 5b), or e) iPSC proteins, i.e. proteins upregulated in iPSCs (Supplementary Fig. 5c). Groups were based by defining differently expressed proteins (DEPs) in the different cell types and the protein set that was uniformly expressed in all cell types. We computed the DEPs as log fold > 1 to select for upregulated proteins, and the top 50 of the sets are given in Supplementary tables 2–4 and are plotted in Supplementary Fig. 7. Of note, the p-values in this figure should be interpreted with caution, since the dependencies of the proteins in the current analysis could not be corrected, leading to an underestimation of these p-values. The overlap in expressed proteins by the different donors is illustrated by Venn-Diagrams in Fig. 3 c and d (days 60 and 115 respectively). The vast majority of the proteins is expressed in the iPSC-derived astrocyte samples of the three donors, showing a high replication of the proteome between samples. The remaining proteins are either specific for each donor or shared by only 2 out of 3 donors, further showing the presence of donor specific variability.

First, we compared the CV across all donors for whole proteome at different stages (i.e. iPSC, day 60 astrocytes and day 115 astrocytes). The average CV did not increase as the differentiation protocol proceeded (r^2 = 0.34, adjusted r^2 = 0.07, n.s. ANOVA), confirming that variability is stable throughout the differentiation protocol. However, the latter results must be interpreted with caution, since the samples size for this particular regression is small, giving too low power to detect statistically significant differences between groups. Furthermore, to statistically test if a particular set of protein is accountable for variability between clones (fibroblast, iPSC or astrocyte associated DEPs), we tested the CV of cell type associated proteins against the non-cell type associated protein set. Since the latter protein set includes proteins that are uniformly expressed among all cell types, this set was used as a reference. This showed that specific protein subsets were significantly more variable than our reference protein set at iPSC and D60 astrocytes. This was largely abolished at day 115 (Supplementary table 5). In turn this shows that proteins driving variability are not identical across donors nor across timepoints.

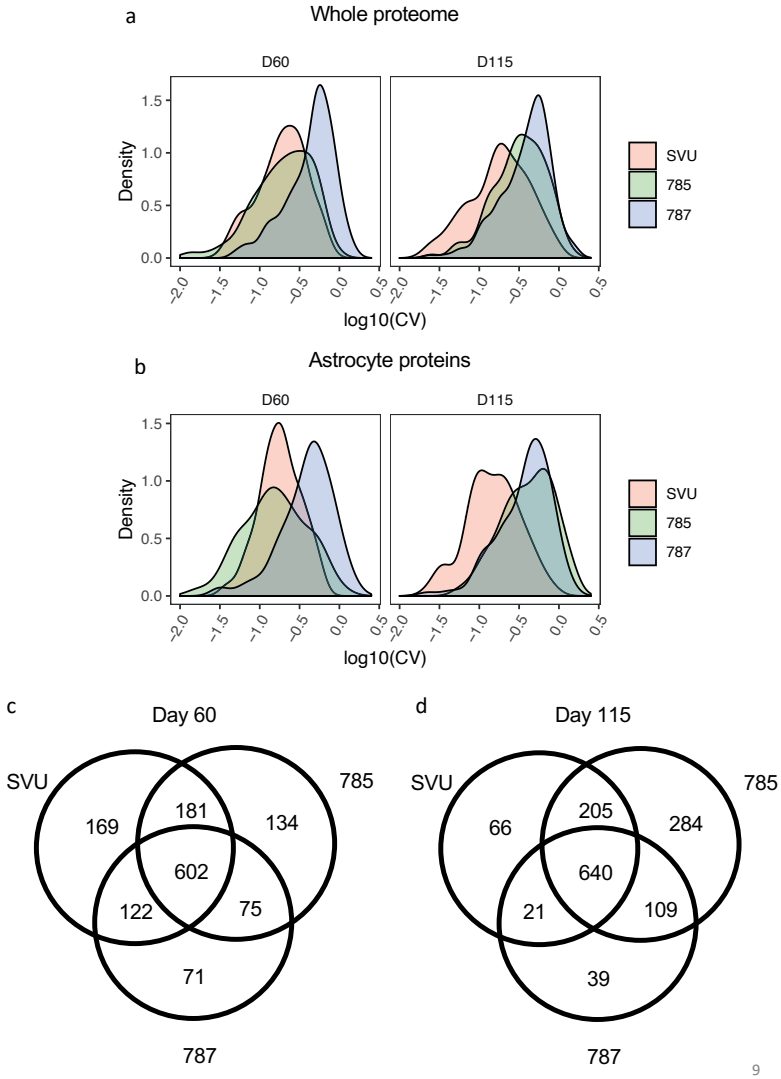


Figure 3: At astrocytes differentiation day 60 and day 115, the coefficient of variance (CV) is studied for each individual, per cell type specific protein group. **a)** whole proteome, **b)** astrocytes. **C)** Venn-diagram showing the overlap in expression of proteins between the different donors at day 60, **d)** and at day 115. Venn-diagrams only include proteins that were expressed by at least one donor's iPSC-derived astrocytes.

To test whether the same proteins are driving variability (regardless of cell type specificity) between (i) donors and between (ii) cell types, we computed the correlations between the CV for the whole proteome of different samples (Fig. 4). Since the correlations between

CVs of different donors are low, our data showed that different proteins can lead to different amounts of variability in the different donors. Furthermore, the correlations do not change throughout the culturing process (Fig. 4 a-b), meaning that the overlap in proteins accounting for the variability between donors remains relatively stable. These correlations between the CVs from different cell types tends to be low (Fig. 4c), indicating that different proteins tend to lead to variability at different stages throughout the protocol. To test whether the top 10% proteins with the highest CV can be annotated, we performed a Gene Ontology (GO) analysis. We showed that proteins with the highest variability at the astrocyte differentiation level were involved in (i) Extracellular structure organization, (ii) Extracellular matrix and, (iii) Proteinaceous extracellular matrix. Overall, this indicates that no specific proteins could be identified that are accountable for variability between samples, but often involve extracellular matrix proteins

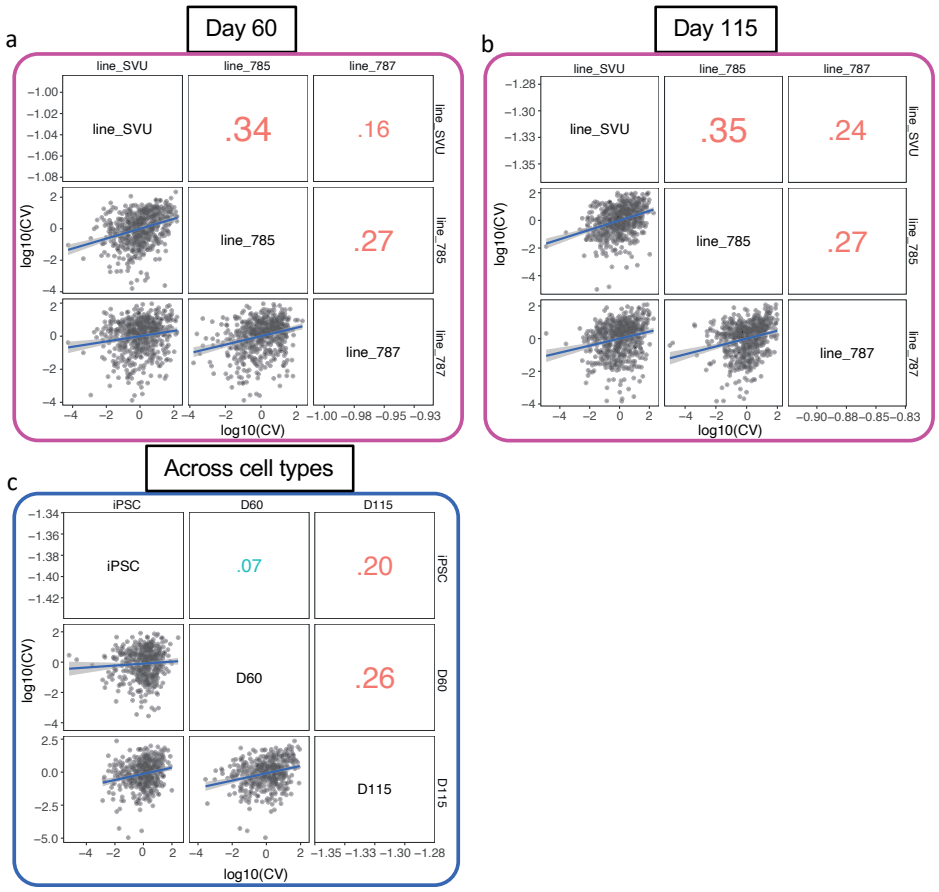


Figure 4: Similarity of coefficient of variance (CV) across lines/ cell type. Pair-wise Spearman correlation for the coefficient of variance of all proteins at day 60 and 115 (**a-b**) and between timepoints (**c**).

Maximizing statistical power with limited sample sizes

A power simulation was set up to investigate how the ratio of independent cell lines vs. clones per cell line influences statistical power, given the correlation structure we observed in our experiment. The purpose of this was to determine whether it would be more effective to increase the number of donors (but limit the number of clones), or to use fewer donors with more clones each, when the goal is to increase statistical power to detect experimental effects (such as differences in morphology, electrophysiological properties or differentially expressed genes or proteins between experimental conditions). For this simulation we used a standard case/control design with a continuous outcome measure, and evaluated the power to detect a difference in group means. In order to simulate the correlational structure reported above, we used a hierarchical linear model in which the outcome of each sample was determined by its cell line membership and case/control status (creating a stronger correlation within cell lines and case-control groups than across) plus some random noise. To determine the relative influence of the number of donors vs clones, we simply varied the total number of donors and clones analyzed (1–15) while fixing the mean difference between cases and controls to 2 and the residual variances for donors to 1.6 and clones to 0.4. This reflects a scenario whereby case/control status explains a total of 33% of the variance in sample means, with an intradonor correlation of 0.8 (see online methods for details).

As expected, our results indicate that increasing the number of donors, i.e. independent cell lines, has a far greater influence on power compared to increasing the number of clones per donor (see Fig. 5). In a hypothetical set-up where 12 is the maximum number of samples per case-control group, one could choose between combinations of 4 donors with 3 clones each or 12 subjects with just 1 clone each. At the current effect size, taking the first option would give us a power of ~60%, whereas the second would give us just over 90% chance of detecting an effect. From this simulation we conclude that although the addition of clones can be beneficious, increasing the number of donors would result in a substantially greater gain in statistical power.

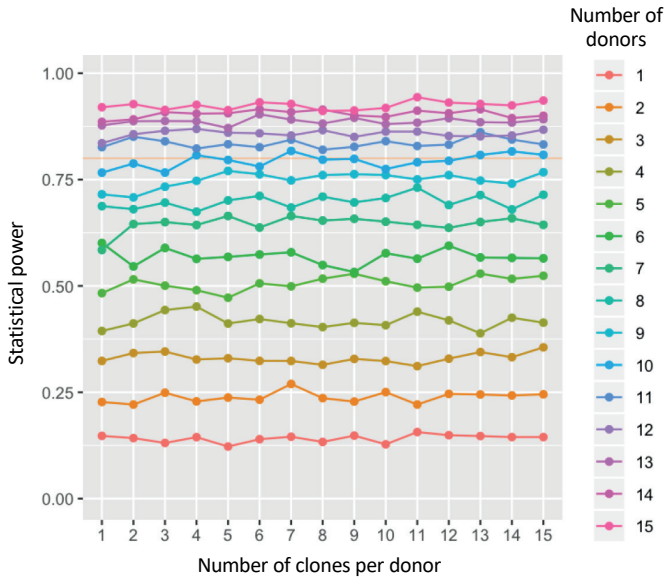


Figure 5: Power simulations based on the variability seen at whole proteome level. Statistical power benefits from including multiple clones per donor when samples sizes are low. Furthermore, the gain in power of adding multiple clones, is never as large as adding more individuals to the groups.

DISCUSSION

The aim of the current research was to characterize the variability between cell lines used in iPSC research and to identify the sources thereof, thereby providing researchers with a guideline of how many clones per donor should be used in order to obtain robust results. We confirm previous findings that genetic background is the largest source of variability, but for the first time based on cellular proteomedata generated by an in-house parallel study design. We show that cells differentiated from iPSC of the same donor are significantly more correlated to each other than to culturing cells from different donors differentiated for 2 months. This suggests that after 2 months of differentiation, the impact of genetic background is significantly larger than differences induced by the reprogramming and differentiation of the iPSCs. With this, our results do not support previous claims of relative high variability between clones. Moreover, analyzing variability using CVs of protein expression suggested that different proteins were driving the variability between clones of different donors.

Our results are in line with several previous studies investigating variability in iPSC research at RNA level (Vitale, 2012, Kyttälä et al., 2016, Carcamo-Orive et al., 2017, Rouhani, 2014). Kyttala et al. (2016) also reveal that both DNA methylation and gene expression of different iPSC lines (microarray) tend to cluster according to the donor, even when clones are derived

from different types of somatic cells. This suggests little variability between clones. However, just like in the current study, some lines from Kyttala et al. clustered with the wrong donor. This is further corroborated by Vitale et al. (Vitale, 2012), who compared 16 different lines derived from 8 subjects and concluded that the variability between clones is small when cells are fully reprogrammed. However, in the principal component analysis performed in their study not all clones clustered together. This interclonal variability remained stable even after random and directed differentiation of the clones, which was partially confirmed by our results. Laurent et al. (Laurent et al., 2011) show that reprogramming often leads to deletions whereas duplications are more prone to emerge by long-term culturing. We could not confirm that occurrence of genetic mutations is causing a decrease in similarity between clones. Our CNV calling did not reveal any *de novo* CNVs that spans a gene that is detected at the protein level in our sample. Furthermore, the CNVs called in the current sample could not explain clustering 'errors' in our unsupervised hierarchical clustering. This is supported by previous findings reporting none or few CNVs in iPSCs that did not deviate from normal mitotic mutation in adult human somatic cells (Tapia and Schöler, 2016, Soldner et al., 2009, Cheng et al., 2012, Kwon, 2017). These authors show that 50% of the genetic variation was already present in the donor tissue (Tapia and Schöler, 2016, Cheng et al., 2012). Claims of genetic instability have further been disproved by Kwon and colleagues, who found only a small number of *de novo* variants in iPSCs compared to donor fibroblasts (Kwon, 2017). Together, these results shows that iPSCs are genomically stable and that this is unlikely to be source of variability in iPSCs.

Another potential explanation for differences between clones is the aberrant hypermethylation reported by several studies, which seems to decrease over time in culture (Tapia and Schöler, 2016, Nishino and Umezawa, 2016). In turn, this means that culturing period will also affect variability measures, complicating the quantification of this type of variability. In general, if this hypermethylation is indeed causal for the 'errors' in clustering of clones and if this hypermethylation is indeed decreasing over culturing period, one would expect that the variability would decrease over time. However, the higher similarity between clones compared to donors seen in our results at day 60 were abolished at day 115. Moreover, the similarity within clones at iPSC stage was larger than at astrocytes day 115. This would suggest an increase in variability throughout culturing period, rather than a decrease as expected when caused by hypermethylation. On the contrary, the similarity of the biological replicates seems to slightly increase over time (from day 60 to day 115). Furthermore, this loss of statistically significant difference in similarity between day 60 and 115 was a result of increase inter-individual similarity rather than due to a decrease of interclonal similarity alone, which would be in line with the previous findings that hypermethylation decreases after long term culturing. Another possible explanation for these seemingly contradictory findings between day 60 and day 115 is the maturation rate. As lines can mature at different

speeds, irrespective of their source, thereby presenting different protein expression profiles at intermediate stages, at the final maturation stage they will mainly present interdonor differences. This will lead to an increase in correlation between donors and thus loss of significance in the comparison against the intraindividual differences. Across time, the significance between the variability between the cell type specific protein sets is abolished, as shown in Supplementary Table 5. The latter suggests that silencing of fibroblast and iPSC genes and the expression of astrocyte genes can vary between iPSC clones and donors, although we should be cautious with interpreting the cell type specific proteins. Together it indicates that analysis at intermediate differentiation stages could be challenging, especially for studies involving low sample numbers, as expression profiles are quite dynamic.

Previous research performed by Bock et al. (Bock et al., 2011) shows that epigenetic and transcriptional variability was mainly driven by a subset of genes (13% and 20% of all genes, respectively) and that most genes did not show large deviations. Furthermore, Swartsentruber et al. (2018) report that genes of importance for neuronal functioning of their sensory neurons were among the most variable ones. In the current study, our CV analysis shows that no cell-type specific proteins particularly account for the variability between samples. Moreover, the correlation of the CVs between cell types was low, which suggests that different proteins account for the variability in different cell types. Furthermore, the interdonor correlation between the CVs of the proteins was stable between day 60 and 115, suggesting an overlap between the proteins causing the variability between clones at both stages. This was not constant for all donors nor for the different time points. The proteins leading to most of the variability seem to be most different between donors at day 60, however, the magnitude of the CVs overall was constant across cell types, suggesting that the latter does not change over time. The variability seems to be driven by genetic background rather than by culturing as also supported by the high similarity within biological replicates and within clones from one donor. Furthermore, GO term analysis of these proteins indicates they are associated with (i) Extracellular structure organization, (ii) Extracellular matrix (ECM) and, (iii) Proteinaceous extracellular matrix. These terms are likely astrocyte specific as they are important in their functioning (Wiese et al., 2012) and because the astrocytes are the primary donors of the extracellular matrix in the brain. Of note, the astrocytes are cultured onto Geltrex coated plates, which could form a potential source for the variability in the ECM proteins. To avoid this would affect our results, we cultured all astrocytes on the same batch of Geltrex.

We showed a decrease in interdonor correlation in our day 115 astrocytes compared to the primary fibroblasts. Reprogramming methods and differentiation protocol can introduce variability. The choice in reprogramming methods can impact the variability between iPSCs (e.g. lentivirus used by Vitale et al and Sendai virus used by Kyttala et al.). This idea is in line with previous reports showing that 80% of the variation in gene expression between

hESCs and hiPSCs is due to residuals of integrative reprogramming methods (Cheng et al., 2012). Removing these residuals significantly reduced variability between lines, posing reprogramming methods as a possible candidate to explain variability. These factors can lead to variability between iPSC clones can in turn compromise their use in disease modeling, especially for subtle phenotypes. However, we have previously performed RNA-seq on iPSCs derived from episomal and lentiviral reprogramming and we did not observe statistically significant differences in variability between the two (Hinz et al., 2019). On the other hand, we have shown that X-chromosomal reactivation can happen after lentiviral reprogramming, and does not happen after episomal reprogramming, which could be accompanied by other epigenetic differences. Also previous reports show that reprogramming methods can impact the transcriptomeof iPSCs (Churko, 2017).

The differentiation protocol of choice probably also impacts the variability between iPSC lines. Protocols generating multiple cell types can lead to higher variability due to differences in cell type ratios. Furthermore, different cell types also express different genes, increasing the chance that CNVs will have a larger impact on gene expression in such cultures. Also, the simplicity of the protocol with regards to the different media and supplements might have an influence on variability, especially when cells are not cultured in parallel or are handled by different researchers or even in different laboratories. Therefore, the translation of our results for optimal study design will depend on the factors as discussed above.

In the current study we show that genetic background of the donors is the largest source of variability in iPSC-derived cells. Our results are based on proteome changes in an in-house parallel design study involving multiple donors, clones and biological replicates. Our power simulations show that using multiple clones per donor has a more noticeable impact than the use of more clones per donor. This is in line with the findings of Germain & Testa (Germain and Testa, 2017), who showed that when using appropriate tests (correcting for dependency) the sensitivity and false discovery rate (FDR) are not improved compared to using one clone per donor. Furthermore, our results are also supported by Aarts et al. (Aarts et al., 2014) who show that optimization of study design benefits from independent observations rather than more observations from one research subject (Aarts et al., 2014). We therefore argue that researchers should opt for large sample sizes (multiple donors) rather than multiple clones. It is important to mention that researchers should in this case invest in extensive characterization of the iPSC lines. This includes CNV calling and confirming whether lines are fully reprogrammed, since large or many CNVs and partial reprogramming have been shown to lead to higher variability. Although, as shown in the current study, the impact of the CNVs in loci that are not expressed in the cell type of interest might not lead to increased variability. Furthermore, we would like to emphasize that the influence of using multiple clones

will depend on the interclonal correlation. When this correlation increases, the addition of multiple clones can be beneficial if an increase in patient numbers is not feasible.

ONLINE MATERIALS AND METHODS

Subjects

The IRB of the Karolinska Institutet approved use of donor fibroblasts (MID 23974872). One additional healthy control fibroblast line was obtained from one anonymous, unidentifiable donor which exempts this line from Institutional Review Board approval. All experiments were exempt from approval of the Medical Ethical Toetsingscommissie (METC), Institutional Review Board of the VU university medical center. A list of all samples included in the current study is given in Supplementary table 1.

iPSC generation

Fibroblasts from the three subjects were expanded, reprogrammed and maintained as described by Hinz et al. (2017). In brief, fibroblasts were cultured and expanded in fibroblast medium (DMEM-F12, 20% FBS, 1% NEAA, 1% Pen/Strep, 50 µM β-Mercaptoethanol). For reprogramming, fibroblasts were dissociated from the plate using Trypsin-EDTA (0,05%, ThermoFisher) where after cells were washed once in PBS and $4x10^5$ cells per desired well were centrifuged (1200 rpm for 5 min). Cells were resuspended in Gene Pulser® Electroporation Buffer Reagent (BioRad) containing 23,3 µg of the reprogramming plasmids (each; Addgene #27078, #27080, #27076). 400 µl per desired number of wells was used for the resuspension and cells were transferred to electroporation cuvettes and were electroporated using the Gene Pulser II (BioRad). Three pulses were generated per cuvette (settings: 1,6kV, 3µF and 400 Ω). This was followed by plating the cells onto Geltrex®-coated plates in fibroblast medium without antibiotics, with 10 µM ROCK-inhibitor (Y-27632; SelleckChem), overnight. Medium was changed into normal fibroblast medium the day after and was refreshed every 3 days until cells reached a confluence of 60–70% (usually around day 5). Medium was then switched to TeSR™-E7™ (STEMCELL) and was refreshed daily. Generated colonies were picked manually and plated onto Geltrex®-coated (Life Technologies) plates containing Essential 8™ medium (Life Technologies) with 10 µM ROCK-inhibitor (Y-27632; SelleckChem) and were refreshed daily (without ROCK-inhibitor).

iPSC characterization for pluripotency

After at least 10 passages, three clones per donor were selected and tested for pluripotency using 1) immunocytochemistry (see section Immunocytochemistry) with antibodies against: OCT4 (C-10, Santa Cruz, 1:1000, mouse), TRA-1–60 (Santa Cruz, 1:200, mouse), TRA-1–81 (Millipore, 1:250, mouse) and SSEA4 (Developmental Studies Hybridome Bank, 1:50, mouse); 2) RNA analysis (see section RNA isolationand PCR analysis) using RT-PCR primers to detect pluripotency markers (listed in Table S6); 3) Embryoid Body formation as described by Hinz et al. (2019) and involving immunocytochemistry with antibodies against α-Smooth Muscle Actin (Progene, 1:1000, mouse), α-Fetoprotein (R&D Systems, 1:1000, mouse) and β-III Tubulin (R&D Systems, 1:1000, mouse). DNA abnormalities we analyzed using the Infinium Global Screening Array by the Human Genomics Facility (HuGe-F) at Erasmus MC, the Netherlands. This was done, in addition to the cell type confirmation based on the MS data (OCT4 expression, described below in the Proteomics section).

iPSC differentiation into astrocytes

iPSC clones that passed characterization for pluripotency were subjected to astrocyte differentiation. The protocol as previously described by Nadadhur (2018)was used in the current study, with minor alterations. One clone per subject was differentiated three times in parallel (Fig. 1). After reaching passage 10, iPSCs were washed twice with EDTA (0,05%) for approximately 30 s each time and left in PBS until colonies came loose by tapping the plate (5 to 10 min). The fragments were collected in 5 ml PBS, and left in a tube until the fragments sank to the bottom. The PBS was aspirated and the fragments were resuspended in 3 ml Neuro Maintenance Medium (NMM; 1:1 DMEM/F12 + GlutaMAX : Neurobasal Medium, 1x B27, 1xN2, 2.5 µg/ml insulin, 1.5 mM L-glutamin, 100 µM NEAA, 50 µM 2-mercaptoethanol, 1% penicillin/streptomycin) containing 20 ng/ml EGF (Peprotech), 4 ng/ml bFGF (Peprotech), 40 ng/ml T3 (Sigma) and 10 µM ROCK-inhibitor (Y-27632), and transferred to an anti-adhesive plate (Poly 2-hydroxyethyl methacrylate coated). Overnight, embryoid bodies formed and were plated on day 3 onto Geltrex®-coated plates in 3 ml NMM containing 20 ng/ml EGF, 4 ng/ml bFGF, 40 ng/ml T3, 10 µM retinoic acid (Sigma) and 10 µM ROCK-inhibitor (Y-27632). 2/3 Medium changes were performed every other day until day 10. At day 10, 100% of the medium was changed into NMM with 20 ng/ml EGF and 40 ng/ml T3 and 2/3 of the medium was changed every other day until day 18. Cells were passaged when confluent. At day 18, 100% of the medium was changed into NMM without vitamin A with 20 ng/ml EGF and 40 ng/ml T3. 2/3 of the medium was changed every other day until day 42 when 100% of the medium was changed into Astrocyte Medium containing 2% FBS, 1% Pen/Strep and 1% Astrocyte Growth Serum (all derived from ScienCell #1801). Thereafter 50% medium changes were done every other day and cells were passaged at confluence using accutase (Millipore). Astrocyte phenotype was confirmed by the presence of several astrocyte markers at protein level (MS, ICC; see Immunocytochemistry and Proteomics sections).

Immunocytochemistry

Cells were plated and cultured onto Geltrex®-coated coverslips. Cells were washed once in PBS and fixated in 4% paraformaldehyde for 10 min. After washing three times with PBS, 10 min each wash, cells were blocked using PBS with 5% normal goat serum (Gibco®), 0.01% bovine serum albumin (Sigma-Aldrich) and 0.03% Triton-X (Sigma-Aldrich) for 60 min. Primary antibodies (see sections iPSC characterization for pluripotency & iPSC differentiation into astrocytes) were diluted in the same blocking buffer and kept at 4 °C overnight. Cells were washed three times in PBS and secondaries were diluted in blocking buffer and incubated for 90 min at room temperature. SOX 9 (CellSignalling, 1:250, rabbit), CD44(Hybridome Bank, 1:100, mouse) GFAP (DAKO, 1:1000, rabbit and Sigma-Aldrich, 1:1000, mouse), Ezrin (Santa Cruz, 1:250, mouse), Nestin (BD Bioscience, 1:1000, mouse), S100b (ProteinTech, 1:1000, rabbit), Vimentin (Hybridoma Bank, 1:200, mouse) and TJP1 (Zymed, 1:100, rabbit). Secondaries: Alexa Fluor® 488 (ThermoFisher, 1:1000, goat anti mouse or rabbit), Alexa Fluor® 594 (ThermoFisher, 1:1000, goat anti mouse or rabbit). After incubation of the secondary antibodies, cells were washed three times in PBS, and DAPI in PBS was added for 5 min. This was repeated by another three washes with PBS where after coverslips were mounted onto mounting glass (SuperFrost) using Fluoromount (Sigma). Fluorescent images were obtained using a Leica DM500 B Fluorescent microscope and LAS-AF software. Further processing was done using Image J version 2.0.0-rc-43/1.52i.

RNA isolation and PCR analysis

RNA was isolated by standard TRIzol®-chloroform isolation and *iso*-propanol precipitation as previously described (Hinz et al., 2019). Complementary DNA was synthesized using Random Hexamer Primers and SuperScript IV Reverse Transcriptase (SSIV; ThermoFisher) with 0,1 M Dithiothreitol (DTT) and 40 U/µl RNaseOUT (ThermoFisher). 1 µg of RNA was used for the RT-PCR. The mix was incubated at 23 °C for 10 min, then at 50 °C for 10 min and finally at 80 °C for 10 min. cDNA was stored at –20 °C until further processing. Phire Hot Start II DNA polymerase (ThermoFisher) was used to for PCR analysis, which was mixed with Phire Buffer (ThermoFisher), dNTPs (10 mM; ThermoFisher), the primer set of interest (listed in Supplementary table 6) and 1 µl of cDNA. PCR program was as follows: 98 °C for 1 min, cycle: 98 °C for 5 sec, 60 °C for 15 sec, 72 °C for 15 sec, which was finished with 72 °C for 30 sec, and kept on hold at 4 °C (Applied Biosystems™ 2720 Thermal Cycler).

Proteomics

Samples were collected (fibroblasts, iPSCs, day 18, day 42, day 59 and day 115 astrocytes, total of 55 samples) by scraping cells with a cell scraper, transferred to an Eppendorf tube, and centrifuged at max speed for 5 min. Supernatant was aspirated and the pellet was washed with PBS twice. Pellets were stored at – 80 °C until sample preparation.

Sample was solubilized in SDS loading buffer, and run shortly in a 10% SDS-gel until proteins migrated about 1–2 cm into the gel. After fixation and staining with Colloidal Coomassie Blue, gel piece that contained proteins was excised and chopped into small gel blocks. The gel blocks were destained with 50 mM NH_4HCO_3/50% acetonitrile, dehydrated using 100% acetonitrile, and dried in a SpeedVac. The gel blocks were rehydrated in 100 µl 50 mM NH_4HCO_3 containing 10 µg/ ml trypsin and incubated overnight at 37 °C. Peptides were extracted with 0.1% trifluoroacetic acid/50% acetonitrile. Sample was dried using a SpeedVac, and stored at –20 °C until mass spectrometric analysis.
The TripleTOF 5600 + mass spectrometer (Sciex) was coupled to an Ultimate 3000 LC system (Dionex, Thermo Scientific). Peptides were fractionated on a 200 mm Alltima C18 column (300 µm i.d., 3 µm particle size) at a flow rate of 5 µl min^{-1}. Acetonitrile concentration in 0.1% formic acid was increased from 5 to 18% at 88 min, to 25% at 98 min, 40% at 108 min and to 90% at 110 min. The eluted peptides were electro-sprayed into the mass spectrometer with a micro-spray needle voltage of 5500 V. The MS survey scan range was *m*/*z* 350–1250 acquired for 250 ms. The top 20 precursor ions were selected for 90 ms per MS/MS acquisition, with a threshold of 90 counts. Dynamic exclusion was 10 s. Rolling CID function was activated, with an energy spread of 5 eV.

The MS raw data were analysed using MaxQuant 1.5.2.8 (Cox and Mann, 2008). MS/MS spectra were searched against the complete UniProt human proteome (release February 2015) with Cys-S-beta-propionamide as the fixed modification and Methionine oxidation and N-terminal acetylation as variable modifications. Label-free protein quantification with MaxLFQ normalisation (referred to as LFQ intensity) and match-between-runs were enabled, remaining settings were left at default. Protein groups that were reverse identifications, identifications from MaxQuant's contaminant database or lacking quantification data in all samples were removed. Samples that did not pass QC were excluded. The analyses in this study were performed as follows: 2837 proteins were identified based on the LFQ intensity, missing values were filtered from the data (total of 192) which was followed by an additional filtering of proteins that had an intensity LFQ of 0 for all samples (93), potential contaminants (59) and reverse proteins (27). This resulted in a data set of 2467 proteins that was used for further analysis.

Raw MS data can be obtained through safe repository after obtaining permission from the Karolinska Institutet in Stockholm.

Pair-wise similarity between samples based on proteomics data

Similarity between two samples was measured by Spearman rank correlation (ρ) using LFQ intensity of proteins with non-zero expressions in both samples. Therefore, the number of proteins used for each pair of samples varies. Wilcoxon Sum of Ranks tests were used to

determine whether groups of correlations significantly differed from each other (eg. testing the set of correlations between samples within the same cell line against the set of correlations between samples of different cell lines).

Variation of protein expression across clones or donors

To compare variation of protein expression across 3 clones within lines per cell type, coefficient of variation (CV) was computed for each protein per line only when all clones had non-zero expression in a line, since there are only 3 samples per gene per comparison. For astrocyte D60 and D115 samples where there were 3 replicates for one of the clones, an average across replicates was computed first, then CV was computed across 3 clones, so that each clone has one value. For each cell type, we extracted proteins that are assessed in all lines (i.e. all samples showed non-zero expression), resulting 802, 602, and 640 proteins for iPSC, D60 and D115 respectively.

To compare variability of protein expressions across 3 lines within cell types, first average across 3 samples within line was computed. This results in one value per line per cell type for each protein. Then CV was computed across 3 lines using average across 3 clones. Note that for D60 and D115, average of replicates was computed prior to the computation of the average across 3 clones. Only proteins with non-zero expression in all clones were analyzed. Average CV was compared between timepoints using linear regression using time as the predictive value and with addition of two dummy variables.

Genes with top 10% of CV were tested for their enrichment in GO terms obtained from MsigDB v6.2 (Subramanian et al., 2005). Since the number of genes in the data set is small, gene sets >5 genes and >2 genes overlapping with top 10% genes were analyzed. Therefore, 12 sets of top 10% genes (across clones in 3 lines × 3 cell types + across lines in 3 cell types) were tested and enrichment P-value was corrected for each set separately by Benjamini-Hochberg FDR.

Up-regulated proteins in astrocytes, iPSCs and fibroblasts

To identify proteins highly expressed in each of fibroblasts, iPSCs, and astrocytes, we used log2 transformed LFQ intensity (with pseudocount 1) and computed the standardized log fold change for each protein as the mean difference in log-intensities divided by their standard deviation. Proteins were considered upregulated in a cell type if the standardized log fold change was>1. These were only computed for the 1,836 proteins which had non-zero expression in at least 50% of samples per cell type in at least one of the cell types.

SNP array and CNV calling

DNA from fibroblasts, iPSCs and astrocytes was isolated after washing cells once with PBS, where after Lysis Buffer (Tris, EDTA, NaCl, SDS) was added with 100 µg/ml Proteinase K and lysate was transferred to an eppendorf tube. Lysate was left in a thermomixer at 37 °C, 600 rpm overnight. Further isolation was performed using phenol/chloroform/isoamylalcohol (25:24:1) (LifeTechnologies), sodium acetate and Ethanol. Obtained DNA was diluted to a concentration of 50 µg/ml. Samples were analyzed using the Infinium Global Screening Array by the Human Genomics Facility (HuGe-F) at Erasmus MC, the Netherlands. CNV calling was performed using the iPsychCNV package in R (Bertalan et al., 2015), which combines information about both the Log R ratio and the B allele frequency to reduce the discovery of false positives. CNVs spanning more than 500 kb and containing at least 100 SNPs were plotted and examined visually. CNVs indicated to have been in loci where DNA quality was indicated to be bad, were excluded from analysis.

The genomic identity of related samples was validated by computing the genetic relatedness in plink 2.0 (Chang et al., 2015) (www.cog-genomics.org/plink/2.0/) at a threshold of >90%. Any samples that did not meet this threshold was excluded.

Simulation

The simulation study sought to investigate the relative influence on power between the number independent cell lines and the number of clones per cell line. We used a standard case/control design with a continuous outcome measure and evaluated the ability to detect a difference in group means. Data was generated from a linear mixed model with group means modelled as fixed and cell line and clone means as random:

$$Y_{ij} = X_j\beta + \delta_j + \epsilon_{ij}$$
$$\delta_j \sim N\left(0, \sigma_\delta^2\right)$$
$$\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$$

Here Y_{ij} represents the outcome for a single clone i in cell line j, X denotes case/control status (0,1) and β is the mean group difference. δ and ε are the residuals for the different cell lines and samples within each cell line respectively. Across the simulations, the effect size and residual variance parameters were fixed (β=2, δ_ε^2 = 1.6, σ_ε^2 =0.4) and only the number of clones and cell lines were varied (the reason being that these parameters do not affect the influence of the sample size ratio on power, but simply scale the effect). The current parameter settings reflect a scenario whereby case/control status explains about 33% of the variance in sample means, with an intradonor correlation of 0.8.

The number of clones and cell lines ranged from 1–15, and data for each possible combination of samples was generated 5000 times. For each run, a corresponding null scenario was simulated by setting β=0. This was done in order to generate a distribution of mean differences under the null, against which the significance of the case/control mean differences was evaluated (p<.05).

AUTHOR CONTRIBUTIONS

S.D.B.H. performed all cell culture and molecular biological experiments with exception of the mass spectrometry, and wrote the manuscript. K.W. performed the quality control of mass spectrometry samples, analyzed the data and contributed to writing the manuscript. J.W. performed the CNV calling, the power simulation and contributed to writing the manuscript. C.A.L. also performed statistical analysis of the proteome data. I.P. performed the mass spectrometry. K.W.L. processed mass spectrometry output. F.K. gave input into quality control and analysis of the data. A.B.S. provided input to writing of the manuscript. D.P. and V.M.H. conceived and supervised the project. All authors reviewed the manuscript.

Acknowledgements

This work was funded by The Netherlands Organization for Scientific Research (NWO VICI 453-14-005). We thank Patrick Sullivan for providing us with two control lines from The Sweden Schizophrenia Study. The Sweden Schizophrenia Study was supported by the Swedish Research Council and US NIMH (awards D0886501 and MH077139 to PF Sullivan).

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde

R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380.e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J,

White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity . Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684 . https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across

4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of "Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human

Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B, Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

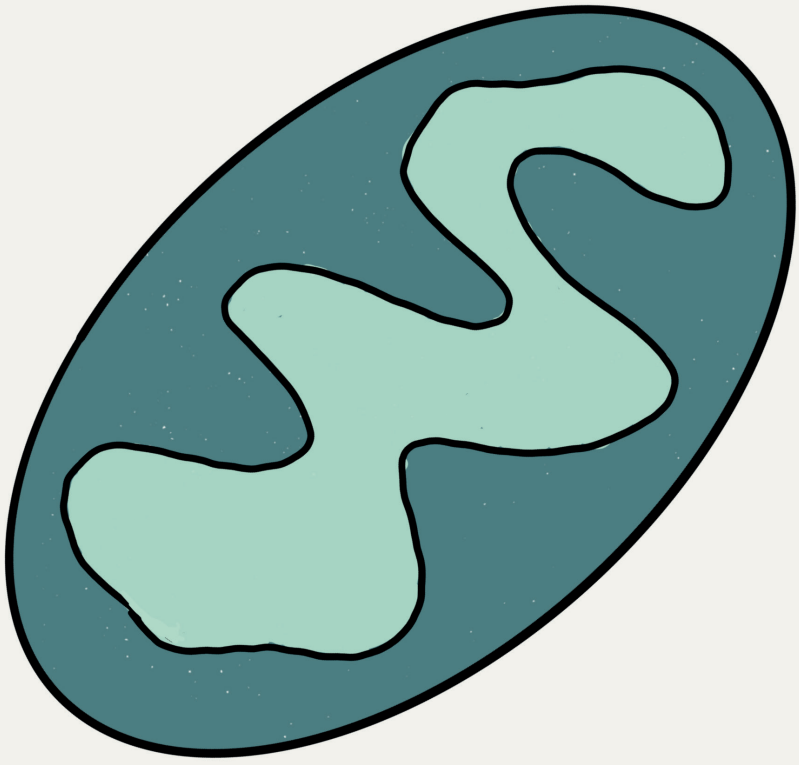
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Chapter 5

iPSC-derived astrocytes with high polygenic risk reveal novel candidate for convergent effects in schizophrenia

S.D. Beekhuis-Hoekstra[1], K.E. Laupman[1], Y.Wei[1], K. Watanabe[1], P. Cornelissen[1] D. Posthuma[1,2] & V.M. Heine[1,2]

[1]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, The Netherlands.

[2] Department of Child & Adolescence Psychiatry, Amsterdam UMC, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands.

Manuscript under consideration in Schizophrenia Bulletin

ABSTRACT

Schizophrenia is a highly heritable developmental disorder with devastating effects on patients and their families. The causal mechanisms behind SCZ remain largely unknown, even after more than a century of research. Astrocytes are increasingly being associated with neuropsychiatric disorders including schizophrenia, yet have gained little attention in research. Therefore, in the current report, we investigate the role of astrocytes in schizophrenia by generating iPSC-derived astrocytes from 21 cases and 10 controls. Moreover, cases and controls were selected using a genetically informed strategy to increase statistical power of our study without a need for a priori knowledge of causal pathways. In addition, we selected patients with different genetic risk factors to test the generalization potential of our results. Testing for transcriptional differences highlighted 10 genes that separated cases from controls. Of these, differences in *SLC25A18* expression were most pronounced. *SLC25A18* is involved in synaptic differences which we observed in our astrocyte-neuron co-cultures between cases and controls: neurons co-cultured with schizophrenia astrocytes show higher VGLUT/VGAT ratios than neurons co-cultured with controls astrocytes. Of note, *SLC25A18* is encoded in a region that has been repeatedly associated with SCZ and its protein transports glutamate across the inner membrane of mitochondria. Our results confirm the involvement of astrocytes in schizophrenia pathology with a specific role for *SLC25A18*, and show that astroglia can indeed create neuronal abnormalities previously reported in SCZ (i.e. synaptic imbalance). More specifically, these findings support previous reports on the involvement of glutamate buffering by astrocytes and mitochondrial defects in the disease etiology of schizophrenia.

INTRODUCTION

Schizophrenia (SCZ) is a severe neurodevelopmental disorder that is characterized by symptoms as hallucinations (positive symptom), social withdrawal (negative symptom) and deficits in working memory (cognitive symptom)(Marder and Cannon 2019). There is no consensus on the causal mechanisms behind SCZ, which hampers identification of new therapeutic methods. Modern technology has confirmed altered connectome of schizophrenic brains (Collin et al. 2016), and different biological mechanisms have been suggested to underlie such dysconnectivity, including white matter abnormalities (Marder and Cannon 2019) and excitation/inhibition imbalance (Sullivan and O'Donnell 2012; Canitano and Pallagrosi 2017; Foss-Feig et al. 2017). Affected glutamate homeostasis has been extensively investigated, as NMDA antagonists rapidly induce SCZ-like symptoms in healthy individuals (Krystal et al. 1994). Moreover, altered expression of its main ligand glutamate have been reported in live imaging of patients (reviewed in Poels et al. 2014) and the role of glutamatergic neurons is supported by genetic evidence (Ripke et al. 2018). More recently, mitochondrial variants have been associated with SCZ and SCZ-associated nuclear variants have been shown to interact with mitochondrial DNA (Gonçalves et al. 2018). In summary, the causal pathways in SCZ have not yet been identified and further research must be performed to shed light on the biological mechanisms leading to this disorder.

SCZ is a symptomatically and genetically complex disorder taunted by heterogeneity, which may be the reason why the causal pathway(s) of SCZ remain(s) unclear. This in spite of the clear genetic component in SCZ: heritability estimate of SCZ is 81% (Sullivan et al. 2012). The genetic variants driving this heritability have remained largely unidentified until the publication of (Consortium 2014). This was by far the largest Genome Wide Association Study (GWAS) at the time. In this GWA study, the authors report the association of SCZ with hundreds of single nucleotide polymorphisms (SNPs). Individually these SNPs have small effects on disease, but the SNP-based heritability (h^2) has currently been estimated to be 0.24 (Trubetskoy *et al.,* 2022). Copy number variants (CNVs, also known as rare variants) are also associated with SCZ and can have larger effect on disease outcome, but are generally rare and explain only a small percentage of cases. CNVs are often also associated with other disorders and they are far from fully penetrant (Kirov et al. 2014). Penetrance and disease outcome of CNVs have previously been shown to depend on additional variants (SNPs) carried by individuals (Tansey et al. 2016). Taken together, the lack of fully penetrant variants and the associations of these with other disorders make it hard to claim causality in SCZ (Hoekstra et al. 2017). Therefore, it would be beneficial to use a disease model that includes the full scope of these variants.

Induced pluripotent stem cells (iPSCs) provides researchers with a new tool to model diseases in vitro on disease-relevant human tissue. A great advantage of iPSCs is indeed the inclusion of the complex genetic architecture of polygenic disorders such as SCZ, as iPSCs are genetically identical to the donor. Since their advent, several iPSC-based SCZ models have been presented. Most of these models have a focus on neuronal dysfunction, including pre- and postsynaptic deficits (Park et al. 2010; Brennand et al. 2011; Wen et al. 2014; Siegert et al. 2015; Schrode et al. 2019; Cleynen et al. 2020), alterations in maturation (Sawada et al. 2020) and excitation inhibition imbalance (Sawada et al. 2020; Kathuria et al. 2020). Interestingly, others also reported deficits that are not neuron-specific (Topol et al. 2015; Casas et al. 2018; Robicsek et al. 2018; Li et al. 2019)or are specific for other cell types, such as astroglia (Toyoshima et al. 2016; Windrem et al. 2017; Akkouh et al. 2020; Szabo et al. 2021; Akkouh et al. 2021)

Indeed, an increasing number of recent studies from different fields within neuroscience (i.e. genetics, MRI/DTI, post mortem) found evidence for glia involvement in the pathogenesis of SCZ. Genome wide association studies (GWAS) have shown that gene sets specific for glia cells (both astrocytes and oligodendrocytes) are associated with SCZ (Goudriaan et al. 2014). Furthermore, MRI studies of SCZ show that cases have smaller white matter volume and can have more potholes in the white matter compared to controls (de Vrij et al. 2019). DTI imaging have linked fractional anisotropy decreases in the anterior cingulate cortex to symptoms severity (Ohtani et al. 2014). Furthermore, postmortem studies have shown abnormalities in astrocytes and oligodendrocytes (reviewed in Ohtani et al., 2014). These glial abnormalities were confirmed by iPSC studies conducted on astrocytes and oligodendrocytes. When glia precursors were injected into immunodeficient shiverer mice or shiverer mice organotypic slices, OPCs migrated less into the white matter (Casas et al., 2018) which resulted in decreased myelination (Casas et al., 2018; Goudriaan et al., 2014). Astrocytes on the other hand, matured less into GFAP+ cells with reduced complexity (Casas et al., 2018). Furthermore, Toyoshima et al. (2016) report, unlike the Goldman group, an increased amount of GFAP positive cells in their mixed differentiations and Akkouh et al. (2020) report attenuated astroglial response to inflammatory activation in cases. Together, these results confirm that astrocytes may play a causal role in the etiology of SCZ, however their exact mechanisms remain unclear.

In the current study we investigate the role of astrocytes in the pathology of SCZ using genetically stratified cases and controls in order to increase statistical power (i.e. cases with high PRS, cases with a relatively penetrant CNVs and controls with low PRS; (Hoekstra et al. 2017). This strategy allows generalization of our results as the latter are not dependent on one single genetic component. Here, we report transcriptional differences between astrocytes from cases and controls in 10 different genes. The top hit *SLC25A18*, located in the 22q11

locus and repeatedly associated with SCZ, also showed altered protein expression. Upon co-culture with SCZ astrocytes, healthy neurons showed a decrease in VGAT positive synapses relative to VGLUT and synaptophysin puncta. Furthermore, we show that increased glutamate levels can alter VGAT positive puncta in our cultures. These findings suggest that affected astrocyte functions in glutamate homeostasis could play important roles in neuronal dysconnectivity in SCZ and should be considered in astrocyte-targeted strategies.

RESULTS

SCZ iPSCs differentiate into astrocyte lineage cells with normal morphologies

Astrocytes have repeatedly been shown to alter neural networks. Moreover, Windrem *et al* (2017) showed that SCZ astrocytes impact the behavior of mice. To further explore what astrocytic mechanism that could underlie neural network dysfunction in SCZ, we selected 10 patients with SCZ who have a significantly increased genomic burden, 5 cases with 16p11.2 duplication, 6 cases with 3q29 deletion and we have chosen 10 controls with low PRS for SCZ (summarized in Fig.1A). iPSCs derived from all patients and controls were differentiated towards astrocytes using a previously published protocol (Tcw et al. 2017; Leferink et al. 2018; Szabo et al. 2021; Beekhuis-Hoekstra et al. 2021) To test for successful differentiation into the astrocytic lineage and potential difference between control and SCZ lines, we processed differentiation day 90 cells for immunocytochemical (ICC) labeling with astrocyte-associated markers CD44, SOX 9, ID3 and NESTIN (Fig. 1B). We used Columbus software to assess different morphological parameters, including cell area (Fig. 1C), roundness (Fig. 1D) and perimeter (Fig. 1E; the latter is the outer border of the astrocyte divided by the area, which gives an impression of the level of protrusions an astrocyte has). All cultures showed typical morphologies for astrocytes and no morphological differences between SCZ cases and control were found as measured by CD44 labeling (T-test, $p>0.05$) (Fig. 1B). In addition, all SCZ cultures presented normal expression levels of the astrocyte-associated markers. Considering previous iPSC-based reports on SCZ showing alterations in neuron/glia ratios in SCZ-derived differentiations (Toyoshima et al. 2016; Windrem et al. 2017), we tested if such skewness would also be present in our sample. However, SCZ iPSCs spontaneously differentiated into mixed neuronal and glial cell populations did not result in changed neuron-glia ratios as measured by astrocyte marker GFAP and neuronal marker MAP2 (Fig 1F-G). Together, we successfully generated astrocyte lineage cells from 21 SCZ cases and 10 control iPSC lines, and showed no overt difference in astrocyte morphologies and marker expression between selected patient and control lines.

A

Diagnosis	Genotype	Sample Size
Control	Low PRS	10
SCZ	High PRS	10
SCZ	16p11.2 duplication	5
SCZ	3q29 deletion	6

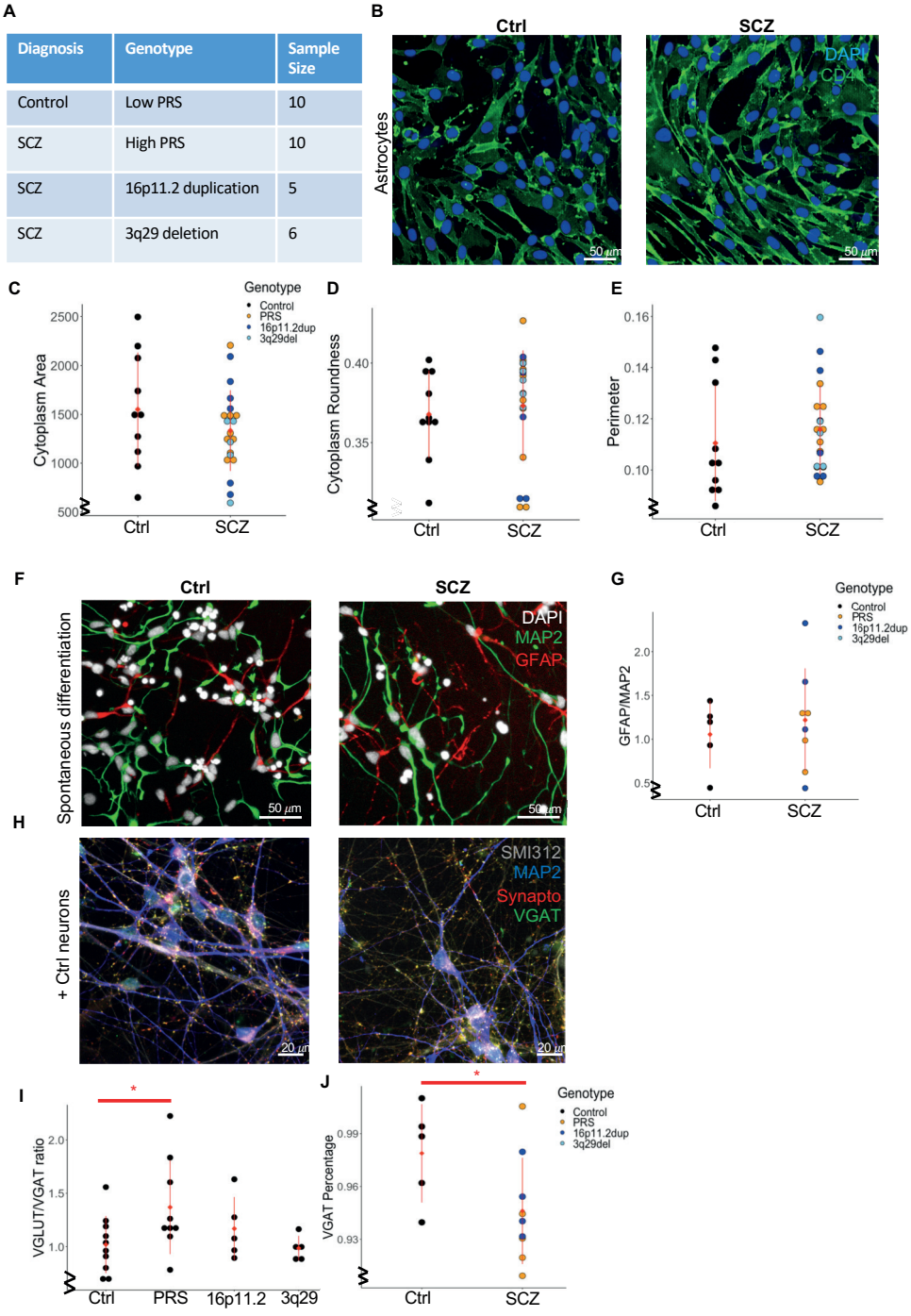


Figure 1: A) overview of subjects includeed in the current study. **B)** Representative images of CD44 immunostained astrocytes from cases and controls. Case and control astrocytes were quantified for: total astrocyte area **(C),** cytoplasm roundness **(D)** perimeter **(E)**. **(F)** Representative images of GFAP and MAP2 after spontaneous differentiation for cases and controls. **(G)** Quantification of the GFAP and MAP2 for all subjects. **(H)** Representative images of SMI312 (axons), MAP2 (dendrites), synaptophysin (post-synapses) and VGAT (inhibitory pre-synapses) after astrocyte-neuron co-culture. **(I)** Quantification excitatory/inhibitory synapse ratios (VLGUT/VGAT) **(J)** Quantification of the percentage of inhibitory synapses (VGAT/synaptophysin) in percentage.

SCZ astrocyte mediate a synaptic imbalance in neuronal networks

To test if SCZ astrocytes affect neural network development, we created neuron-glia co-cultures and studied the glutamatergic/GABAergic synapse balance. To avoid neuronal batch difference could influence the results, all SCZ and control astrocyte lines were co-cultured with the same batch of iPSC-derived neurons as previously described (Nadadhur et al. 2018). After 35 days of co-culture, all cultures were immunoassayed for MAP2, VGLUT and VGAT followed by automated microscopic scanning and automated analysis using Columbus software (Fig.1H). Upon the quantification of the number of VGLUT- and VGAT-puncta per MAP2-positive dendrites, we calculated the VGLUT/VGAT ratio for each cell line. ANOVA comparison between each individual group revealed no statistically significant difference in VGLUT/VGAT ratios. However, by directly comparing the high PRS cases to the low PRS, which we selected to increase statistical power (Hoekstra et al 2017), we found a significant decrease in the VGLUT/VGAT ratio in the cases (T-test $p < 0.05$; Fig. 1I). Such significance was not reached for cases carrying 16p11.2 duplication or 3q29 deletion (Figure 1I). Interestingly, when we studied whether a decrease in VGAT expression, as a percentage of the total number of synaptophysin, could underlie VGLUT/VGAT ratio changes, all SCZ cases did show significant changes (Fig. 1J $p < 0.05$, t-test). Such differences in synaptic ratios were not seen when SCZ and healthy neurons were cultured on rodent astrocytes (supplementary figure 1). This suggests that SCZ astrocytes can induce VGLUT/VGAT ratio changes in control neurons.

Differential gene expression in genetically burdened SCZ astrocytes

To investigate transcriptional differences between SCZ and control astrocytes, we performed RNA-sequencing analysis on all iPSC-derived astrocytes from cases and controls. A Wald-test of controls vs. cases (all cases grouped) revealed significant downregulation of 10 genes in SCZ after correction for multiple testing (*SLC25A18, GPM6B, CEND1, MAPK10, CD74, ZNF436, NEFL, FAM43A, HS6ST1, PTN, APOE*; Table 2). The differentially expressed genes are ranked from most to least significantly altered transcripts (Table 1A). A likelihood ratio test was performed separating the SCZ cases with different genetic risk factors (PRS vs 3q29 vs 16p11.2 vs low PRS controls), but did not reach statistical significance after multiple testing correction. However, a post hoc Wald-test between groups (Table 1B) resulted in

Table 1: Differential gene expression analysis between SCZ patient and control (Ctrl) iPSC-derived astrocytes.

Gene	Protein name	*Wald test between Ctrl vs. SCZgroup*			*Wald test per genotype*	
		baseMean	**log2Fold Change**	**p-value adjusted**	**per genotype**	**p-value adjusted**
SLC25A18	Mitochondrial glutamate carrier 2	66.88	-3.14	*0.005*	Ctrl vs. PRS Ctrl vs. 16p11.2	*0.002* *0.012*
GPM6B	Neuronal membrane glycoprotein M6-b	619.68	-3.84	*0.006*	Ctrl vs. PRS Ctrl vs. 3q29 Ctrl vs. 16p11.2	*0.003* *0.018* *0.009*
CEND1	*Cell cycle exit and neuronal differentiation protein 1*	49.96	-2.97	*0.006*	Ctrl vs. PRS Ctrl vs. 16p11.2	*0.001* *0.025*
MAPK10	*Mitogen-activated protein kinase 10*	65.26	-2.54	*0.006*	-	*n.s.*
CD74	*HLA class II histocompatibility antigen gamma chain*	544.68	-4.32	*0.026*	-	*n.s.*
ZNF436	*Zinc finger protein 436*	871.25	-1.91	*0.033*	-	*n.s.*
NEFL	*Neurofilament light polypeptide*	485.66	-2.78	*0.042*	-	*n.s.*
FAM43A	*Protein FAM43A*	214.78	-2.09	*0.043*	-	*n.s.*
HS6ST1	*Heparan-sulfate 6-O-sulfotransferase 1*	1160.02	-1.91	*0.044*	-	*n.s.*
PTN	*Pleiotrophin*	877.40	-2.60	*0.044*	-	*n.s.*
APOE	*Apolipoprotein E*	699.31	-1.03	*n.s.*	Ctrl vs. 16p11.2	*0.011*
RARRES1	*Retinoic acid receptor responder protein 1*	57,35	0.44	*n.s.*	Ctrl vs. PRS PRS vs. 3q29	*0.024* *0.009*
SERPINB9	*Serpin B9*	151.26	0.72	*n.s.*	Ctrl vs. 16p11.2 PRS vs. 16p11.2 3q29 vs. 16p11.2	*0.005* *0.001* *0.040*
TDRD7	*Tudor domain-containing protein 7*	116,74	-0.03	*n.s.*	Ctrl vs. 16p11.2 PRS vs. 16p11.2	*0.000* *0.000*

A) Wald-test analysis showed significant down regulation of 10 transcripts in SCZ astrocytes when Ctrl cases were compared to all SCZ cases. **B)** To test differential gene expression between Crtl and SCZ per genotype i.e. polygenic risk score (PRS), 3q29 and 16p11.2, a LRT test with post hoc Wald analysis was performed. LRT did not reveal any significant changes, however, to illustrate the distribution of expression between groups, we have performed post hoc testing regardless of the lack of statistical significance. Red represents down and green represents up regulation. Expression analysis was performed on control versus case samples with DESeq2 and was corrected for multiple testing using Benjamin Hochberg false discovery rate correction. Samples with a corrected p-value of 0.05 or lower were defined as differentially expression genes (DEGs).

one gene that was consistently altered across all cases: *Glycoprotein M6-b (GPM6B*). *Solute Carrier Family 25 Member 18* (*SLC25A18*) was only downregulated in cases with high PRS and in cases with the 16p11.2 duplication, but was not down regulated in cases with a 3q29 deletion. The same was seen for the *Cell Cycle and Neuronal Differentiation 1 (CEND1)* gene. *GPM6B* and *CEND1* are both involved in neuronal differentiation and *GPM6B* expression was also observed in astrocytes after injury (Choi et al 2013). *CEND1* is involved in cell cycle exit (Politis et al 2007).

Increased GC2 expression in SCZ

The most significant downregulated transcript is *SLC25A18* that translates into protein mitochondrial glutamate carrier (GC2) (adjusted p=0.005; Table 1). GC2 is one of the two glutamate / H+ symporters in mitochondria (Fiermonte et al. 2002), and is involved in glutamate uptake in astrocytes. Glutamate is a neurotransmitter that is crucial for proper neuronal functioning and is associated with SCZ (Poels et al. 2014). Also, *SLC25A18* is encoded on loci 22q11.21 (Footz et al. 2001), just before the start of the CNV associated with DiGeorge syndrome, a variant that has repeatedly been associated with SCZ. To determine whether altered transcript levels in SCZ vs. control also translates to protein level, we performed a western blot analysis of its protein GC2. We compared the expression of 6 controls and 9 cases, which revealed a statistically significant difference in CG2 expression ($p<0.05$, t-test). However, the difference in GC2 expression was opposite of what we expected based on the RNAseq data (Figure 2A-B). SCZ astrocytes show significantly increased protein expression in mitochondrial glutamate carrier GC2, together with changes in SLC25A18 transcript, this suggests that SCZ astrocytes might have impaired functions in glutamate homeostasis.

Glutamate concentration alters synaptic network

To show a causal link between increased GC2 expression and the synaptic imbalance, we overexpressed GC2 in human primary astrocytes using lentiviral infection (pCMV(pr) SLC25A18-IRES2-EGFPlenti). As shown by western blot, the astrocytes infected with the *SLC25A18* construct showed increased expression of GC2 compared to control astrocytes (pCMV(pr) IRES2-EGFPlenti) (Figure 2C). To test whether GC2 overexpression in astrocytes results in synaptic imbalance, we cultured GC2-transduced astrocytes with iPSC-derived human neurons. We indeed show a decrease in VGAT positive puncta as shown in Fig. 2D. However, since the experiment was only performed in one cell line, we cannot statistically test this synaptic phenotype. Furthermore, we hypothesized that altered glutamate metabolism could underlie the synaptic alterations induced by SCZ astrocytes. We tested this hypothesis by adding L-glutamic acid to our iPSC-derived neuron-astrocyte cultures. Automated analysis of our synaptic ICC as described above, revealed that the percentage of VGAT positive puncta (relative to synaptophysin puncta) is decreased when glutamate is added (Fig.2E) and this effect was much more pronounced in the cultures with astrocytes that overexpress

GC2. Together these results suggest that GC2 can indeed lead to differences in synaptic balance, which is likely mediated through glutamate clearance.

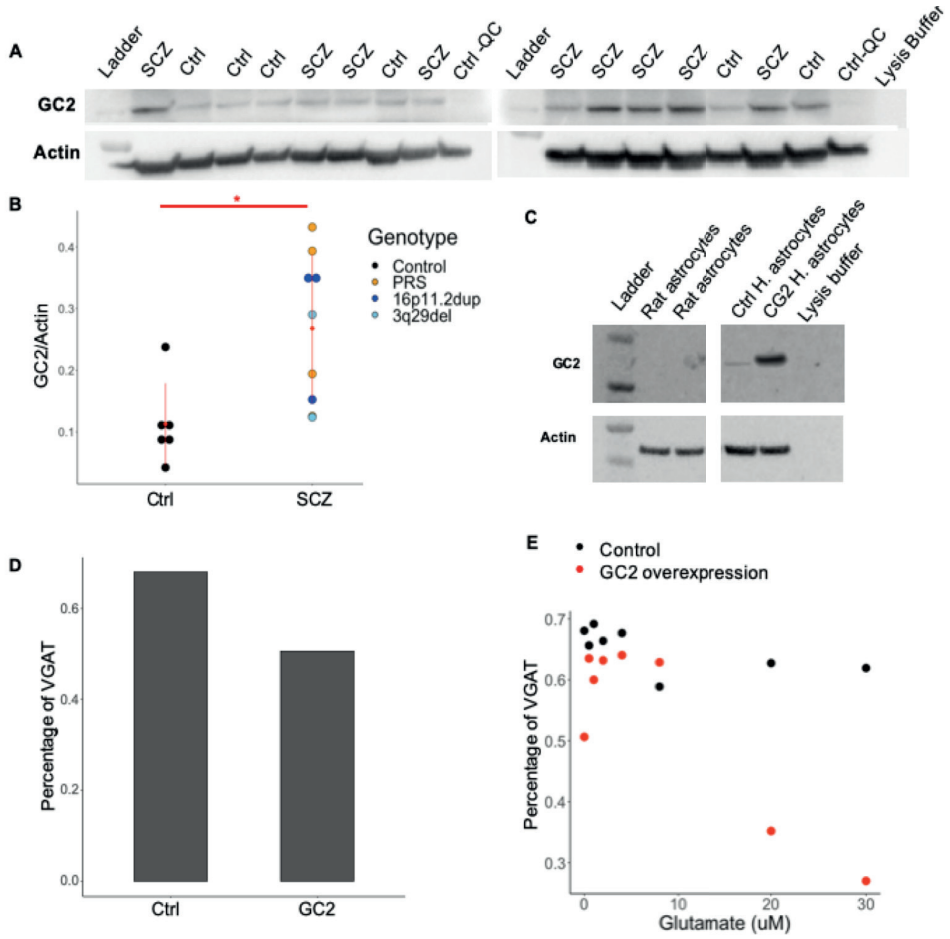


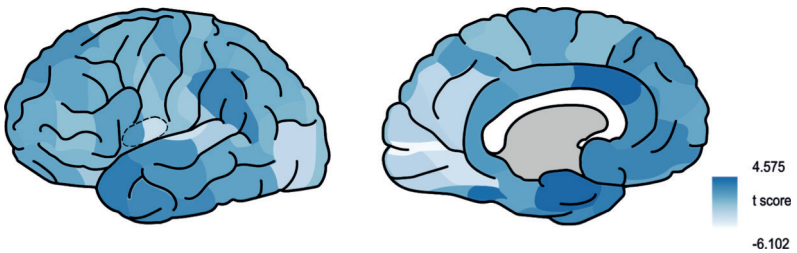
Figure 2: The influence of GC2 and Glutamate on synapses. **A)** Western Blot experiment for the expression of GC2 protein in control and SCZ samples. **B)** Quantification of the western blot results dispicted in pannel A.**C)** Western blot results after GC2 overexpression with lenti viral incorporation of the human *SLC25A18* gene. **D)** Quantification of the VGAT positive synapses in neurons co-cultured with ctrl astrocytes and GC2 overexpressing astrocytes. **E)** Quantificatin of the VGAT percentage in neurons co-cultured with ctrl astrocytes and GC2 overexpressing astrocytes upon long term glutamate stimulation

SLC25A18 enrichment in the anterior cingulate cortex

Since the expression *SLC25A18* is altered in SCZ astrocytes, we investigated the expression of this gene in the healthy human brain, using transcriptomics data from the Allen Human Brain

Atlas (see Online Methods). This included data from the left hemisphere of six postmortem healthy human brains. We compared expressions of *SLC25A18* in tissue samples within the region to the rest of the tissue samples for each Desikan-Killiany region using two-sample t-tests. This revealed significantly up-regulated gene expression in caudal anterior cingulate, entorhinal, fusiform, medial orbitofrontal, supramarginal, and temporal pole ($q < 0.05$, FDR corrected; one-sided; significant brain regions are listed and visualized in Figure 3A-B). The largest effect size was observed for the caudal anterior cingulate (Cohen's $d = 1.12$). Combining the caudal anterior cingulate and the rostral anterior cingulate showed statistically significant upregulation of *SLC25A18* within the anterior cingulate cortex compared to the rest of the brain ($t = 4.87$, $p < 0.001$; Figure 3C). The observed effect size exceeded null distributions of effect sizes obtained from random genes (one-sided $p = 0.048$, 10,000 permutations). In addition, comparing expression levels of *SLC25A18* to null distributions of expression levels of random genes showed a marginally significant over-expression for *SLC25A18* in tissue samples within the anterior cingulate cortex (one-sided $p = 0.047$, 10,000 permutations). These results suggest a preferential expression of *SLC25A18* in the anterior cingulate cortex of the human brain, revealing a relevant expression pattern of this gene in SCZ pathology.

A



B

Brain Region	T score
Caudal anterior cingulate	4.57
Entorhinal cortex	4.50
Fusiform area	4.11
Temporal pole	3.05

C

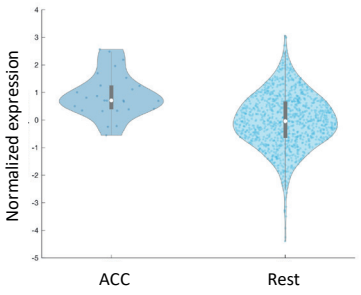


Figure 3: GC2 Expression of SLC25A18 in postmortem brain (Allen Human Brain Atlas). **A)** Visualization of expression levels of SLC25A18 in the human cortex. **B)** Desikan-Killiany region with significantly

enhanced expression of SLC25A18. Combining the caudal anterior cingulate and the rostral anterior cingulate showed statistically significant upregulation of SLC25A18.

DISCUSSION

In the current study, we investigated the role of astrocytes in the etiology of schizophrenia. We analyzed astrocytes in isolation and in co-culture with neurons. Astrocytes in mono-culture did not reveal any morphological differences between cases and controls. Neither did proteins involved in maturation and astrocytic identity, which was previously reported in SCZ iPSC-derived cells (Toyoshima et al. 2016; Windrem et al. 2017). However, when SCZ astrocytes were co-cultured with control human neurons, the ratio of VGLUT/VGAT positive puncta increased, in line with multiple reports showing an E/I imbalance in SCZ (Foss-Feig et al., 2017; Canitano et al., 2017; Sullivan & O'Donnell, 2012). Our results further suggest that the difference in ratio between VGLUT and VGAT comes from a decrease in the inhibitory synapses, rather than an increase of the excitatory synapses. This also confirms previous reports that loss of inhibition is a feature of SCZ (Shao et al. 2019) and that astrocytes play an important role in neural network alterations in SCZ; which is also in line with Liu et al (2019) who showed that abnormal differentiation of astrocytes was accompanied by a downregulation of potassium channels, involved in hyperexcitability (as also shown by the authors). The identification of altered expression in *SLC25A18* and in its protein GC2, a mitochondrial glutamate carrier, in SCZ astrocytes, is suggesting a prominent role for glutamate metabolism and mitochondria in SCZ pathomechanisms, and so provides a connecting link between earlier posed hypotheses. Thus, we now confirmed that the lack of inhibition, which has been repeatedly shown in SCZ, can be -at least partially- caused by astrocytes.

Using RNA-seq we showed a decrease in expression of 10 transcripts in astrocytes generated from cases. Interestingly, we report a decrease of several genes involved in stress pathways (*PTN*, *MAPK10*, *GPM6B* and *CD74*). This contradicted our expectations, as stress is thought to be one of the triggers of SCZ onset. However, another study using iPSC-derived SCZ astrocytes reported decreased expression of stress related genes (Akkouh et al. 2020) in line with our current results. The top hit in our sample, *SLC25A18,* was only significantly altered in the high PRS cases and the 16p11.2 duplication carriers and not in the 3q29 deletion carriers, showing a difference between cases depending on their genetic background. The same was seen for gene *CEND1*. *GPM6B* was significantly altered in all cases, regardless of genetic background. The latter two genes are involved in neuronal differentiation, which would imply skewed differentiation as previously reported (Toyoshima et al. 2016; Windrem et al. 2017). However, as shown, we did not find such differences. Though we did not study this extensively and cannot exclude that another differentiation procedure could result in skewed differentiation into the astrocyte cell lineage.

In contrast with our RNA-seq findings, the expression of the protein GC2 is increased in SCZ astrocytes rather than decreased and this increase was consistent across all tested SCZ patients. A possible explanation for the discrepancy between the findings of the RNA-seq and the western blot experiments is given by Topol et al (2015), who report an increase in mRNA translation in SCZ cases. They report an increase of ribosomal proteins and translation initiation factors and a global increase of 64-69% of proteins, including mitochondrial proteins. Furthermore, such discrepancy was also reported by another group investigating mitochondrial genes in SCZ (Bar-Yosef et al. 2020a). These results together with the current results, suggest that protein levels cannot always be estimated from mRNA expression, and should be quantified directly. This illustrates the need for the validation of gene expression in molecular research.

GC2 is crucial for the supply of glutamate to the TCA-cycle. Moreover, glutamate is often associated with SCZ, and microdialysis of SCZ patients have reported increased concentrations of glutamate (Eide and Stanisic 2010). We hypothesized that glutamate metabolism might be altered in SCZ cultures and could be the source of the phenotype seen in the current study. This led us to investigate the effects of increased levels of extracellular glutamate on neuron-astrocye co-cultures. We show that the addition of glutamate alters synaptic balance, providing further confirmation for a potential role for the glutamate carrier. In accordance, SCZ iPSC-derived astrocytes have been shown to alter glutamate uptake (Szabo et al. 2021). To evaluate if the synaptic findings can indeed be explained by the overexpression of GC2, we induced GC2 overexpression in astrocytes, following co-culture with neurons. Network formation in the presence of astrocytes overexpressing GC2 indeed yielded a lower percentage of VGAT positive synapses. Together, these findings confirm a potential role for glutamate clearance in astrocytes and synaptic imbalance in SCZ.

As we did not directly investigate potential mitochondrial alterations in our samples, we cannot conclude there are mitochondrial alterations in the current study. However, in support of a pronounced role for mitochondria in the etiology of SCZ, many others have provided evidence from different fields. Post mortem studies showed alterations in mitochondrial shape, localization and functionality (Uranova et al. 2001; Maurer et al. 2001; Karry et al. 2004; Iwamoto et al. 2005; Somerville et al. 2011; Manji et al. 2012; Arion et al. 2015). In accordance, increased glycolysis has been shown, which confirms reduced cellular respiration (Regenold et al. 2012). There is also genetic evidence that nuclear encoded genes for mitochondrial proteins are associated with SCZ (Millar et al. 2005; Park et al. 2010; Gonçalves et al. 2018), and there is evince for the association of mitochondrial variants and this disorder (Schulmann et al. 2019). The latter is supported by the increased maternal inheritance (Verge et al. 2012). More specifically, a duplication of the *SLC25A18* gene is seen in 6 out of 20 cases reported in an isolated Dagestan population (Bulayeva et al. 2016),

providing a more direct link to SCZ. Direct evidence for mitochondrial alterations in cases comes from blood derived mitochondria, showing decreased copy numbers of mitochondrial DNA (Li et al. 2015; Kumar et al. 2018) and mitochondrial dysfunction (Casademont et al. 2007; Bar-Yosef et al. 2020a). Furthermore, there is comorbidity between SCZ and mitochondrial myopathy encephalopathy lactic acidosis (MELAS)(Shao et al. 2008; Anglin et al. 2012). Additional support is given by studies showing that antipsychotic drugs influence mitochondria (Burkhardt et al. 1993; Modica-Napolitano et al. 2003; Casademont et al. 2007; Bar-Yosef et al. 2020a) and studies in mice and *in vitro* show alterations in the transcriptome of mitochondrial proteins involved in the TCA cycle and ATP production (Gokhale et al. 2019; Gordon et al. 2021). Moreover, two different studies investigating SCZ using iPSCs have shown mitochondrial abnormalities in neurons from SCZ patients (Robicsek et al. 2013; Li et al. 2019). In summary, evidence for mitochondrial alterations in SCZ comes from many different fields, and is consistent. We now added to this body of evidence that mitochondrial alterations in astrocytes can affect neuronal networks in a way that also has been consistently been reported in cases: synaptic or E/I imbalance (Collin et al. 2016).

We further showed that *SLC25A18* is preferentially expressed in the ACC. This brain region has emerged as a potential brain area that is affected in SCZ, which has currently also been confirmed by the latest GWAS (PGC 2020). The ACC has elaborate connections to the limbic structures and also receives dopaminergic input from the ventral tegmental area, making this region strategically relevant in SCZ. As discussed, many studies have linked decreased connectivity to psychosis. In support of this dysconnectivity theory, leukodystrophy patients often suffer from psychosis at disease onset and specifically the white matter integrity in the ACC has been linked to psychosis (Marder and Cannon 2019). Also, SLC25A18 is encoded in a genetically relevant locus (directly before the break point of the 22q11.2 deletion) and duplication of *SLC25A18* has been found in several SCZ cases (Bulayeva et al. 2016). In summary, our candidate gene is located in a genetically relevant location, organelle and brain region, making it an interesting candidate for further study its association with SCZ.

In the current study we provide direct evidence that SCZ astrocytes alter neuronal network development with a potential underlying role for glutamate metabolism and mitochondria. Moreover, our results might clarify the many different findings in SCZ etiology. Important strengths of the current study are the relatively large sample size, our genetically stratified sampling method, which does not require a priori knowledge of causal pathways. The latter allowed us to perform an unbiased exploration of potential causal pathways in SCZ astrocytes. Moreover, this sampling strategy also gives us higher statistical power to detect differences between control and affected astrocytes. Our study also poses some limitations, such as the lack of direct evidence for glutamate metabolism and mitochondrial stress. An additional limitation is the commercially available medium used to culture astrocytes contains

fetal bovine serum, which has been shown to alter astrocyte morphology and transcriptome towards reactive astrogliosis (Zhang et al. 2014). Despite the overwhelming evidence for their role in SCZ, future research should elaborate on the link between mitochondrial alteration such as ATP production, glutamate metabolism and dysconnectivity.

MATERIALS AND METHODS

Subjects

Cases and controls were selected based from the Swedish Cohort based on their Polygenic Risk Score (PRS) (Consortium 2014). Ten patients with a PRS within the highest 1% were selected for the current experiments and ten controls were selected with the lowest 1% PRS. Subjects carrying CNVs associated with SCZ were excluded. Furthermore, eleven additional cases were selected based on the presence of wither one of two specific CNVs: a 3Q29 deletion (six patients) or a 16p11.2 duplication (five patients). Skin biopsies of cases and controls were obtained by the Karolinska Institutet (PMID 23974872).

iPSC generation and astrocytes differentiation

Fibroblasts were obtained from the Karolinska Institutet, and expanded onto gelatin coated flasks and in fibroblast medium (DMEM-F12, 20% FBS, 1% NEAA, 1% pen/Strep, 50 µM β-Mercaptoethanol). Reprogramming of these fibroblasts was done as previously described in Hinz et al (2018) and Beekhuis-Hoekstra et al (2020). In brief, fibroblasts were episomally reprogrammed using plasmids #27078, #27080, #27076 from Addgene. Colonies were manually picked and iPSC lines were kept on Geltrex coated plates in Essential 8 medium.

Astrocytes were derived from the iPSCs according to our previously published protocol (Tcw et al. 2017; Leferink et al. 2018; Szabo et al. 2021; Beekhuis-Hoekstra et al. 2021)

Neuronal differentiation

Neurons were derived from 14 lines using a modified version of the protocol published by Nadadhur et al (2017). In brief, iPSCs were differentiated towards neural stem cells (NES cells) using dorsomorphin and 10 µM SB431, for 12 days with daily refreshment of 2/3 of the medium. After these 12 days, neural rosettes were manually picked and cultured onto PLO/mLAM in NMM supplemented with 20ng/ml EGF and 20ng/ml FGF2, with daily refreshment of 1/2 of the medium. Cells were passaged at confluence using TripLE. In the current experiments, passage 3 NES cells were used for further differentiation towards neural precursor cells. When cells reached a confluence of 90%, 50% of the medium was switched to N2 medium supplemented with 400 ng/ml hSHH. Cells were kept in this medium for 4 days with daily medium changes of 50%. This was followed by the switching of the medium to Neurobasal

medium composition supplemented with 10 µM valproic acid. Cells were kept in this medium for 3 days, again with daily medium refreshments. NPCs were dissociated using accutase, and passaged onto PLO/mLAM in Neurobasal medium composition supplemented with 20 ng/ml BDNF, 10 ng/ml GDNF, 10 ng/ml IGF and 1 µM cAMP for 10 days. For suspension cultures this was followed by a second accutase passage where 3 million viable cells were plated onto 12 coverslips (PLO/mLAM plated, 16 or 18 mm in a 12 well plate). Cells were left to attach in Neurobasal medium composition supplemented with 10 µM ROCK-inhibitor, 20 ng/ml BDNF, 10 ng/ml GDNF, 10 ng/ml IGF and 1 µM cAMP. The next day, coverslips were flipped upside down to form suspension culture. For such cultures, 12 well plates were used, where bumps were made at the bottom of the well and where astrocytes were plated (50.000 astrocytes per well for human astrocytes, no coating. Either primary astrocytes were used or iPSC derived astrocytes Day 90). The medium of the astrocytes was replaced by the medium of the neurons. From this day onwards, 50% medium refreshment was performed twice a week using Neurobasal medium composition supplemented with 1 µM cAMP. For cultures with direct contact between astrocytes and neurons, neurons were plated onto 7 000 Day 90 astrocytes on glass bottom 96 wp precoated with PLO/mLam in NB medium supplemented with 10 µM ROCK-inhibitor, 20 ng/ml BDNF, 10 ng/ml GDNF, 10 ng/ml IGF and 1 µM cAMP. After plating, 50% of the medium was refreshed twice a week with NB + 1 µM cAMP. In all conditions, neurons were treated with 2 µM AraC at day 24 and ICC was performed 56 days after neuronal induction (hSHH treatment).

Immunocytochemistry

Astrocytes on coverslips were fixated with 4% paraformaldehyde for 20 minutes. Neurons on glass coverlsips were fixated using ice cold acetone for 10 minutes at -20°C. Neurons in plastic 96 well plates were fixated in ice cold acetone : methanol 1:1 for 10 minutes at -20°C. All conditions were then washed with PBS 3 times. This was followed by an incubation in blocking buffer for 1 hour (PBS, 5% normal goat serum (Gibco), 0.1% bovine serum albumin (Sigma), 0.3% Triton X-100 (Sigma), then by 1 hour incubation at RT in blocking buffer with primary antibodies, which was in turn followed by an overnight incubation at 4°C (list of primary antibodies can be found in supplementary table 1). Coverslips were then washed 3 times with PBS and secondary antibodies Alexa Fluor® 488 (ThermoFisher, 1:1000), Alexa Fluor® 647 (ThermoFisher, 1:1000) and Alexa Fluor® 594 (ThermoFisher, 1:1000) diluted in blocking buffer were incubated for 2 hours at RT. This was again followed by 3 washes in PBS and cells were incubated in PBS with DAPI for 5 minutes, and then washed again. Coverslips were mounted onto superfrost glass using Fluoromount™ (Sigma-Aldrich). 96 well plates were kept in PBS and imaged within 24 hours.

Image analysis ICC

10 images were taken per coverslip with a Leica DM6000B microscope and analyzed using the automated Columbus software, were PNG images were loaded as visual images. 96 well plates were analyzed using the CX7 automated microscope and images were analyzed using the Columbus software. 30 images were taken per well and autofocus was based on DAPI staining.

In all cases, imaging and exposure times were kept constant throughout the imaging of an entire experiment. For neurons, dendrites were mapped based in anti-MAP2 staining. This was followed by the detection of VGLUT, VGAT or synaptophysin puncta specifically on MAP2. For VGLUT/VGAT ratios, the total synaptic counts for VLGUT (on MAP2) was divided by the synaptic counts for VGAT (again on MAP2). This was performed for the total counts of an entire well. VGAT percentages were determined by first tracing synaptophysin puncta on MAP2, then tracing VGAT puncta on the previously found synaptophysin puncta. Percentages were calculated by dividing the counts for VGAT-syanptophysin overlap by the total synaptophysin puncta. Again, this was performed for total counts within one well (thus based on 30 images).

RNA sequencing

Astrocytes were cultured as described above. RNA was isolated from cells using the standard TRIzol®-chloroform isolation and iso-propanol precipitation. Library preparation was done according to Hinz et al (2018). In brief, Agilent 2200 TapeStation system (tape D5000) was used to determine the RNA integrity scores (RIN). All sample pairs that scored 8 or higher were included in the current study. Library preparation was performed with a TruSeq mRNA sequencing kit (Illumina) according to the recommendations by the manufacturer. 100 ng of RNA per sample was loaded for prepping, from which mRNA was purified using poly-T oligo magnetic beads, which was in turn fragmented. SuperScript IV (ThermoFisher) and Frist Strand Synthesis Act D Mix (Illumina) were used for reserve transcription which was followed by production of the second strand (incorporating of dUTP) was done with Second Strand Marking Master Mix (Illumina) and samples were purified. A-tailing and the ligation of the adaptor were performed with Ligation Mix and Stop Ligation Buffer (Illumina), and samples were washed with AMPure XP Beads (Beckerman Coulter). Enrichment of DNA fragments was performed in 15 cycles using PCR Primer Cocktail and PCR Master Mix (Illumina), which was followed by the last purification step with AMPure XP Beads (Beckman Coulter). The quality of the library preparation was determined using Agilent Technologies 2100 Bioanalyzer and samples were pooled for sequencing with the Illumina HiSeq 4000 (50 base pair single read).

Western blot

Astrocytes were cultured in a 6 well plate without coating, in ScienCell medium. Cells were washed 1x with cold PBS on ice, and lysed in RIPA buffer (150 mM NaCl, 1% Triton-X100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, 1mM DTT, protease inhibitor) for 10 minutes where after they were scraped off using a sterile cell scraper, and collected in an Eppendorf on ice. Samples were centrifuged at max speed for 5 minutes and supernatant was transferred to a new Eppendorf tube and stored at -80 °C until further processing. Samples were quantified using the Nanodrop 2000C and Bradford reagent. 50 µg of protein for each sample was loaded for the SCZ samples and 30 µg of protein was loaded for the overexpression samples in an Invitrogen precast gel and western blotting was performed in a Tank Blotter (Biorad) on PVDF membranes (Biorad) as recommended by the manufacturer. Blots were then blocked for 60 minutes in TBS with 0.05% Tween and 5% milk powder at RT. Antibody against *SLC25A18* was incubated in TBS with 3% milk (1:750) O/N at 4°C. Blots were washed 3 times in TBST, where after it was incubated with TBST containing secondary antibody HRP for 2 hours (1:10 000; Dako P0448). Blots were imaged using enhance chemiluminescence West Femto (Thermo Fisher 34095) and Licor scanner. Actin was stained for 1 hour at RT and was visualized using West Pico Plus (Thermo Fisher 34577).

Statistics

Immunocytochemistry data were tested by averaging the results per individual (as several images were taken for each subject), which was followed by statistical analysis using R (version 3.6.2). Comparison of astrocyte morphology, astrocyte maturation (based on the expression of maturation markers), and glial skewness were tested by performing a t-test. The first synaptic counts of our co-cultures were analyzed by performing an ANOVA (separating the cases based on genetic background), which was followed by post-hoc t-test only comparing PRS cases vs. controls. Synaptic comparisons of following experiments were performed using a t-test where all cases we grouped.

Western blot results were analyzed using Fiji (version 2.0.0-rc-43/1.52i) using the gel analysis tool to determine protein expression, where after these results were analyzed by a t-test using R (version 3.6.2). All results were plotted using R (version 3.6.2, ggplot2 package).
RNA sequencing results were analyzed using DESeq2 package in R. There were 28 samples included in the RNA sequencing, of which one sample was excluded from further analyses due to a low read count (< 10 million reads). Batch number was included as covariate in the analyses of differently expressed genes (DEG). only genes with TPM>1 in 50% of samples in all groups were analysed, and the number of tested genes in each analysis are specified below. False discovery rate (Benjamin Hochberg) correction was performed for each analysis and genes were defined with abs(log2FoldChange)>log2(1.5) and adjusted P-value<0.05 as significant. Pairwise comparisons were done with a Wald test (null hypothesis is log2 fold change is equal to 0). For multiple group comparisons, a likelyhood ratio test (LRT) was used.

Gene expression

Microarray gene expression data were obtained from the Allen Human Brain Atlas (http://human.brain-map.org), including data from six postmortem brains of human donors without any neuropathological or neuropsychiatric conditions. Microarray analyses are described in detail in http://help.brainmap.org/display/humanbrain/Documentation. An average of 466 brain tissue samples of the left hemisphere were obtained from four donors (466 ± 72.6 samples from H0351.1009, H0351.1012, H0351.1015, and H0351.1016), and 946 and 893 samples covering both hemispheres from the remaining two donors (H0351.2001 and H0351.2002). We included tissue samples in cortical regions of the left hemisphere and used the expression of 58,692 probes for each brain donor (Wei et al. 2019).

Probe-to-gene re-annotations were performed using the BioMart data mining tool (https://www.ensembl.org/biomart/) {ref}. Per brain, and per tissue sample, expressions of probes annotated to the same Entrez gene ID were further averaged, followed by log2 transformation with pseudocount 1, resulting in gene expression levels for 20,333 genes. Tissue samples were spatially mapped to the FreeSurfer cortical regions from a cortical parcellation of 114 regions (57 per hemisphere) based on the Desikan-Killiany atlas (DK-114) {for detail, see (Wei et al. 2019)}. Gene expressions per gene were normalized to z-scores within each donor across all cortical tissue samples.

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug

treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380.e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen

FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J, White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical

region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity . Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684 . https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic

JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of "Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived

neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data

QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Trubetskoy V, Pardiñas AF, Qi T, et al. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature*. 2022;604(7906):502-508. doi:10.1038/s41586-022-04434-5

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B, Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering

of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

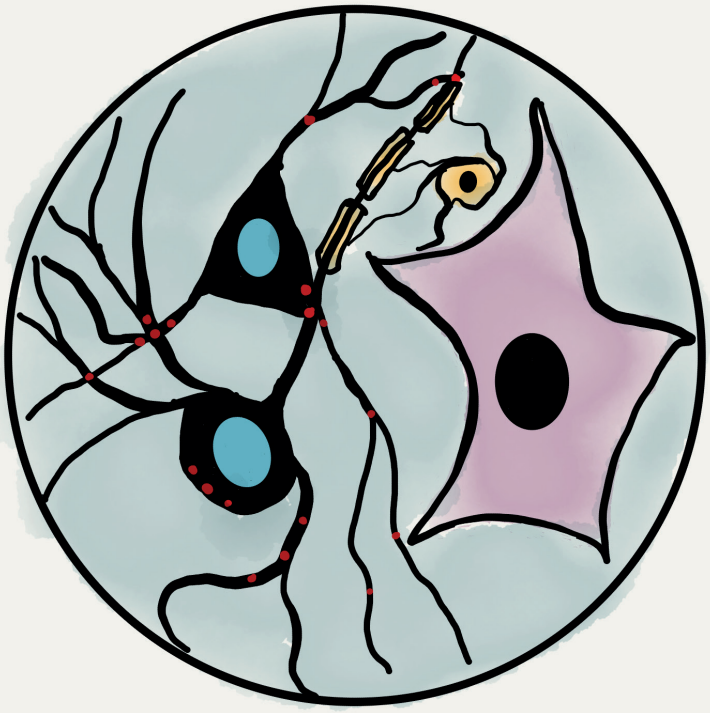
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Chapter 6

Developing a 3-dimensional multicellular model for schizophrenia

S.D. Beekhuis-Hoekstra, S. Hunt, Christiaan de Kock, D. Posthuma & V.M. Heine

ABSTRACT

Schizophrenia (SCZ) is a severe genetically complex neurodevelopmental disorder. The causal biological mechanisms behind SCZ remain unclear. While neurons have received the vast majority of the attention in the past decades, recent reports have revealed potential abnormalities in glia cells and white matter in SCZ pathology. In the current report, we investigated whether a 3-dimensional model consisting of multiple cell types could potentially be used to evaluate synergistic effects of neurons, astrocytes and oligodendrocytes in SCZ. In the current model we show the presence of functional neurons, astrocytes and myelinating oligodendrocytes. This potentiates the investigation of the interplay of several cell types and their potential additive effects.

INTRO

Schizophrenia (SCZ) is a complex neurodevelopmental disorder that is characterized by the presence of symptoms falling into three categories: positive, negative and cognitive symptoms (Consortium et al. 2020). It has a relatively high prevalence in the population (1% worldwide) and it is thought to arise from a combination of genetic a predisposition and early environmental affecters (both during prenatal period and later in life (Marder and Cannon 2019). Moreover, there is consensus that the high genetic component in SCZ is polygenic and genetically complex (Consortium 2014). Despite the long history of SCZ research and the many hypotheses formulated, the causal biological mechanisms of SCZ are yet to be determined. While research for long focused on neuronal dysfunction, there is an increasing body of evidence for the involvement of glia and white matter. Our recent results indicate that the causal biological pathways of SCZ might lie, at least partly, in the interplay between neurons and astrocytes: in Chapter 5 of this thesis we showed that astrocytes can induce synaptic imbalance in neuronal networks, due to a decrease in inhibitory synapses. Other groups also previously reported the involvement of neuron/glia skewness (Toyoshima et al. 2016; Windrem et al. 2017). Interestingly, as oligodendrocytes and myelination of axons have been shown to rely on astrocyte factors and on neuronal activity, the complex neuron-astrocyte-oligodendrocyte interactions pose another potential intercellular interplay that might underlie SCZ.

Induced pluripotent stem cells are a highly suitable method to mimic the genetic complexity of SCZ in vitro, which cannot be achieved in traditional transgenic animal models (Hoekstra et al. 2017). Therefore, the field of neuropsychiatry has developed many iPSC-based model systems. In line with the increasing interest in glia dysfunction in neurological disease, the development of complex neuron-glia model systems, including brain organoids and spheroids, increased in recent years. It is now possible to allow the differentiation of different neural cell types in a 3-dimensional (3D) environment (Monzel et al. 2017). Compared to a 2D model, a 3D organoid model represents a more physiological model, and allows the investigation of the convergent effects of diseased neurons, astrocytes and oligodendrocytes. Therefore, 3D brain organoids might provide new model systems to investigate the additive effects on and of oligodendrocytes in neuron-astrocyte microenvironments.

Here we present a pilot study on the use of brain organoids to investigate SCZ pathophysiology. We used genetically stratified cases and controls to obtain brain organoids that include myelin-forming oligodendrocytes. As described in Chapter 5 of this thesis, we have selected patients based on extremely high polygenic risk as well as the presence of highly penetrant CNVs, which resulted in the inclusion of cases with 3 different backgrounds: high polygenic risk score patients, cases carrying a 16p11.2 duplication and cases carrying a 3q29 deletion

(Chapter 5 of the current thesis). Healthy controls were selected for the absence of any known highly penetrant CNVs for SCZ as well as an extremely low polygenic risk for SCZ. First, to characterize the brain organoids for presence of neurons, astrocytes and oligodendrocytes and their regional identity we performed immunohistochemistry for specific cell type and brain regional markers. Using these results, we could make a timeline of the differentiation process within the organoids. To test for the generation of neuronal activity, we performed patch clamping experiments. Second, we made use of lines from both controls and SCZ cases, to determine whether we can reproduce the synaptic imbalance seen in the previous chapter or previously published phenotypic differences reported in SCZ, such as skewness in cell type and differences in maturation.

RESULTS

Organoids show specification of region-specific neurons and neuronal functionality

In order to investigate the potential involvement of oligodendrocytes in the pathology of SCZ, we adapted an organoid culture that contains myelinating oligodendrocytes (Monzel et al. 2017). Slight alterations were made, such as the starting neuroepithelial stem cells (NES) population which was derived from a previously in-house developed astrocyte protocol, we used human Sonic Hedgehog instead of its agonist purmorphamine, T3 instead of transforming growth factor ß3 and we added IGF1 for additional neuronal support. First, exploration of regional identity of our organoids revealed that they express cortical markers CTIP2 and Reelin (figure1), but they also express genes that are preferentially expressed in the (lateral) ganglionic eminence (MEIS 1&2). Reelin was solely present at the outermost layer of the organoids, while CTIP2 was expressed in the region right below the Reelin-positive outer layer. Furthermore, GAD65/67 immunoreactivity revealed the expression of GABA-ergic neurons in our organoids. MEIS 1&2 and GAD65/67 showed both clusters in earlier time points but were more widespread at later stages. This model therefore includes excitatory and inhibitory neurons.

Timeline analysis of our organoids reveals glia specification

To confirm presence of glial subtypes, we studied the presence of proteins preferentially expressed in astrocytes (GFAP and SOX9) and proteins preferentially expressed in oligodendrocytes (OLIG2 and MBP). Time line analysis of cell type expression within our cultures showed the presence of astrocytes and oligodendrocyte precursors at the first timepoint (day 35 revealed immunoreactivity for GFAP and OLIG2 respectively, see figure 2). GFAP expression was widespread throughout the organoids, while OLIG2 expression at this stage was clustered.

A

Protein stained	Marker for
CTIP2	Cortical layer V and IV
Reelin	Cortical layer I
PAX 6	Neuronal precursor
MEIS 1	Ganglionic eminesce
GAD 65/67	GABAergic neuron

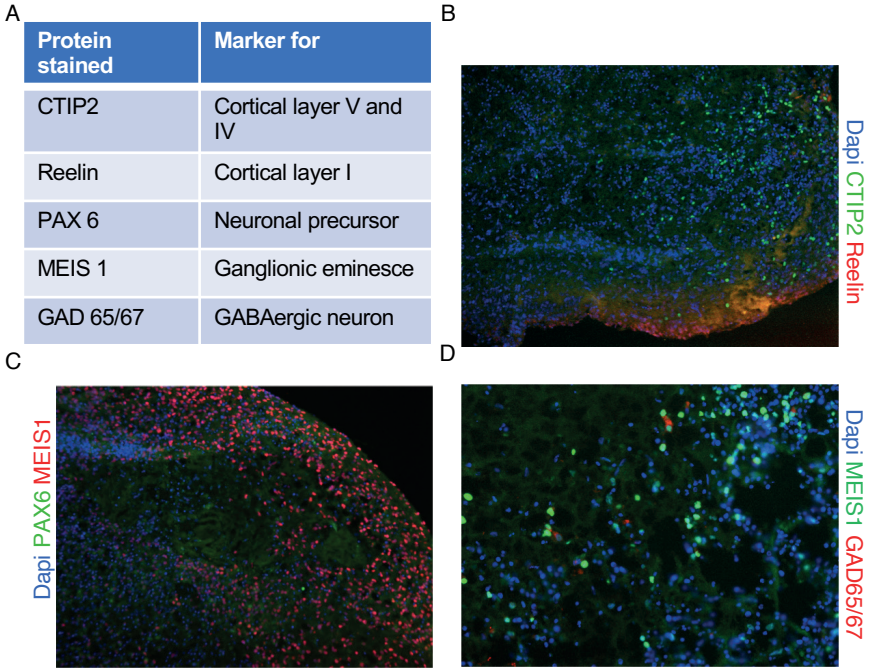


Figure 1: Exploration of cortical markers within our organoids. **A)** List of markers tested. **B)** ICC of CTIP2 and reelin, revealing a presence of layer IV and V neurons, and layer I neurons respectively. **C)** ICC analysis reveals the presence of neuronal precursors (PAX6) and neurons derived from the ganglionic eminence. **D)** GAD 65/67 further shows the presence of GABAergic neurons within our organoids.

Of note, the expression of neuronal specific marker MAP2 was also first shown and widespread at day 35. At this stage, there was no sign of MBP, which indicates that there are no mature oligodendrocytes. Moreover, 35-day old organoids showed broad expression of rosette formation, showing that NES cells in the organoids have self-organizing potential. At differentiation day 60, MBP became visible in a subset of lines, indicating that at this timepoint, OPCs start to reach maturity (figure 2B). This was not the case yet for all cell lines, suggesting that the period around differentiation day 60 might be the crucial period for oligodendrocyte maturation in these cultures. At differentiation day 90, all lines showed broad expression of MBP, suggesting that organoids at this stage are mature enough to form oligodendrocytes that have myelinating potential (figure 2C). Even though MBP and axon marker NF200 co-localization in figure 2C resembles myelination, we have only analyzed the organoids in a 2D image. 2-dimensional imaging cannot be used to study the enwrapping of MBP protein around the NF200-positive axons. Therefore, we are not able to undoubtedly confirm myelination in our brain organoids. Nevertheless, our analysis does confirm the presence of astrocyte- and oligodendrocyte-lineage cells in our model, providing the opportunity to investigate the interplay between neurons (excitatory and inhibitory), astrocytes and oligodendrocytes.

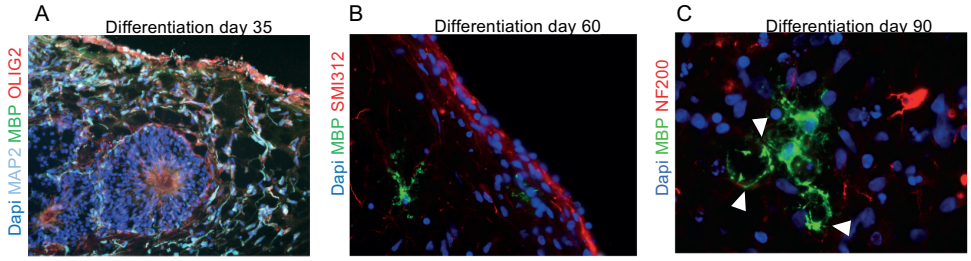


Figure 2: Oligodendrocyte maturation process. **A)** At day, 35, several rosettes were seen per organoid as visualized by the flower-like structure in ICC analysis of the organoids. No MBP or OLIG2 positive cells were present. **B)** At day 60, OLIG2-positive cells became apparent and few MBP-positive cells were detected. **C)** At day 90, more MBP-positive cells we detected, which showed MBP NF200 co-localization. The number of MBP-positive cells varied per slice and per organoid.

No differentiation skewness towards astrocytes

The presence of all neuronal cell types in the current model makes it highly suitable for testing of potential skewness in differentiation tendencies. We previously reported decreased expression levels of GPM6B and CEND1 in iPSC-derived astrocytes from SCZ patients compared to those of controls, but failed to show skewness between astrocytes and neurons in Chapter 5. Here we tested if there was an altered ratio of the neuronal marker NF200 in comparison to the astrocytic marker GFAP in the current model (figure 3). As the organoids are analyzed in cryopreserved sections, which means that cells will be cut and cell fragments will be present scattered over slices, we compared total fragment length per image using Columbus software. This did not show any difference in neuron/glia ratio between cases and controls. In order to compare cell counts, we have stained our organoids with SOX9 and OLIG2 to determine the percentage of astrocytes and oligodendrocyte precursor cells respectively by comparing the OLIG- and SOX9-positive cells to dapi counts per image (Figure 4). In line with GFAP/NF200 analysis, no differences in the percentage astrocytes were detected between SCZ cases and controls. OLIG2 counts also did not reveal any difference between cases and controls. Moreover, SCZ organoids show a very variable percentage of OLIG2. Overall, there was no evidence for a skewed differentiation tendency towards neurons, astrocytes nor oligodendrocytes in our model.

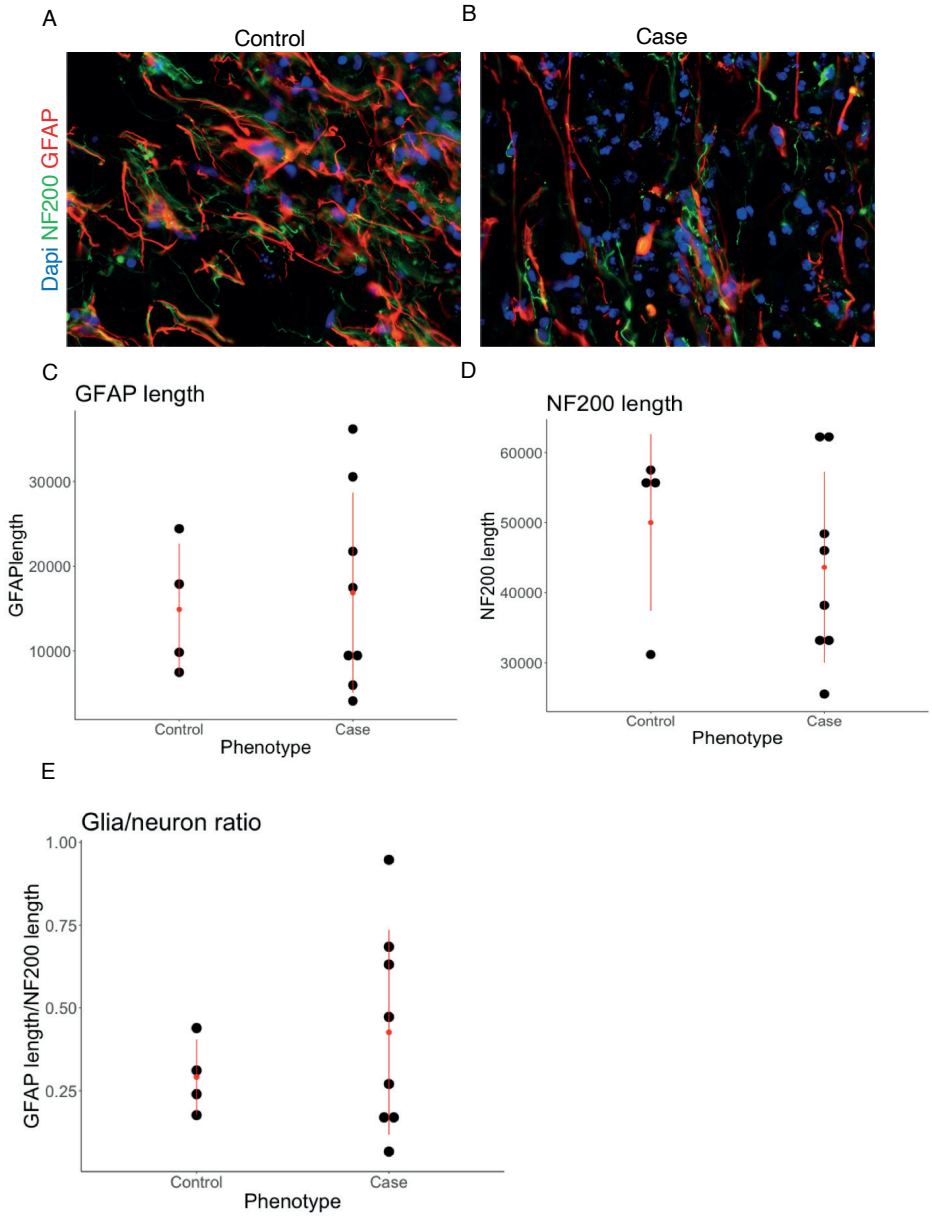


Figure 3: Glia/Neuron ratios. **A)** Representative image of axonal bundles (NF200) and astroglial processes (GFAP) for a control organoids slice. **B)** Representative image of axonal bundles (NF200) and astroglial processes (GFAP) for a high PRS case organoids slice. **C)** Quantification of GFAP expression for cases and controls. Each dot represents the mean sum length of 5 images of one single individual. **D)** Quantification of NF200 expression for cases and controls. **E)** Ratio between GFAP length and NF200 length for cases and controls.

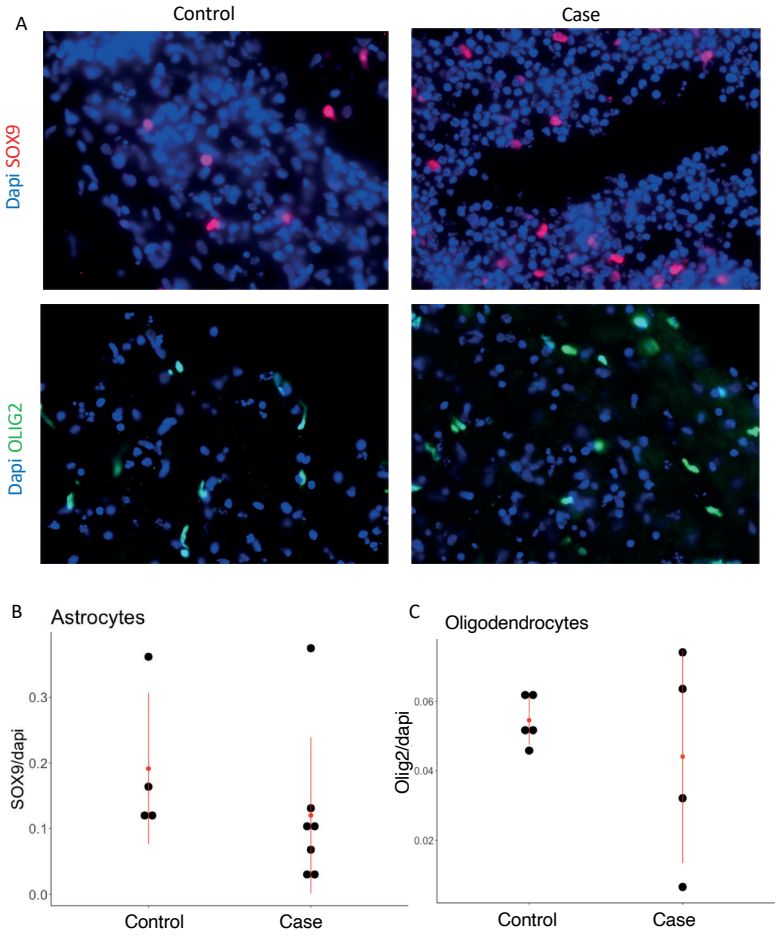


Figure 4: Oligodendrocyte/astrocyte ratios. **A)** Representative image of SOX9 and OLIG2 expression in SCZ cases and controls. **B)** Quantification of SOX9-positive cells in cases and controls. **C)** Quantification of Olig2-positive cells in cases and controls. Each dot represents the mean ratio of 5 images of one single individual

Slower action potentials in homozygous CACNA1C risk allele

To further characterize our organoid model, we tested if the neurons in our organoids are mature enough to generate action potentials. Since our organoids also include glia cells, we performed a lentiviral infection of our NES cells with CFP tag under CAMKII promotor to label glutamatergic neurons (Dittgen et al. 2004) to facilitate the patching of neurons. Organoids were patched in artificial cerebrospinal fluid (aCSF) and organoids from all lines were able to generate action potentials (APs) under electrical stimulation. In this setting, AP threshold, AP amplitude, AP rise-time, AP half-width and AP onset were determined (figure 5). These measurements can be indicative of neuronal functioning and maturation. We compared the electrophysiological properties of controls and SCZ cases. Passive membrane properties of control and patient-derived cells were not significantly different (membrane resistance: control median 1400 Interquartile range (IQR) 1893MΩ, patient median 1100 IQR 1237 MΩ, p = 0.75; resting membrane potential: control median -34.52 IQR 23 mV, patient median -37.22 IQR 14 mV, p = 0.36; figure 5D). Seven of 24 control cells and 11 of 25 patient-derived cells fired APs in response to positive current injection and displayed similar active properties (AP threshold: control mean -35.8 ± 3.7 mV, patient mean -31.3 ± 7.0 mV; p = 0.09 ;AP amplitude: control mean 48.6 ± 9.4 mV, patient mean 37.8 ± 14.4 mV, p = 0.07; AP rise time: control mean 1.0 ± 0.5 ms, patient mean 1.216 ± 0.4, p = 0.44), with the exception of AP half-width, which was significantly faster in control cells (control mean 1.774 ± 0.4 ms, patient mean 2.94 ± 1.7 ms, p = 0.048). More detailed inspection of the AP rise-time revealed that the difference seen between cases and controls was driven by one single line, and might not represent alterations in SCZ patients in general

No excitation/inhibition imbalance

Since we have functional neurons in our 3D model, we next aimed to verify the findings of our previously published 2D neuron-astrocyte co-culture, where we saw an imbalance in excitation/inhibition. More specifically, we saw an increased ratio of VGLUT/VGAT synaptic counts (Chapter 5). Unfortunately, at the time, staining our organoids with these synaptic markers resulted in high background noise, hampering reliable synaptic counts. Therefore, we used PCR for the quantification of synaptic proteins, where we investigated the expression of these markers, together with the expression of GAD65-67, NEUN and GFAP. The VGLUT/VGAT ratio of our organoids were not altered between cases and controls at mRNA level. Furthermore, VGAT/NEUN and VGLUT/NEUN ratios were also not different between the groups. We also tested GDA65-67/NEUN ratio in an attempt to clarify if there is a difference in the GABA-ergic vs. glutamatergic neurons but this did also no show any difference between controls and SCZ. With the current analysis we cannot confirm the presence of a synaptic imbalance in SCZ. Potentially, there is no imbalance in our 3D model, or we did not detect any due to a lack of statistical power due to smaller sample sizes and increased variability of this model. Additionally, imbalances can be found with synaptic counts, which are broadly used to study E/I imbalance.

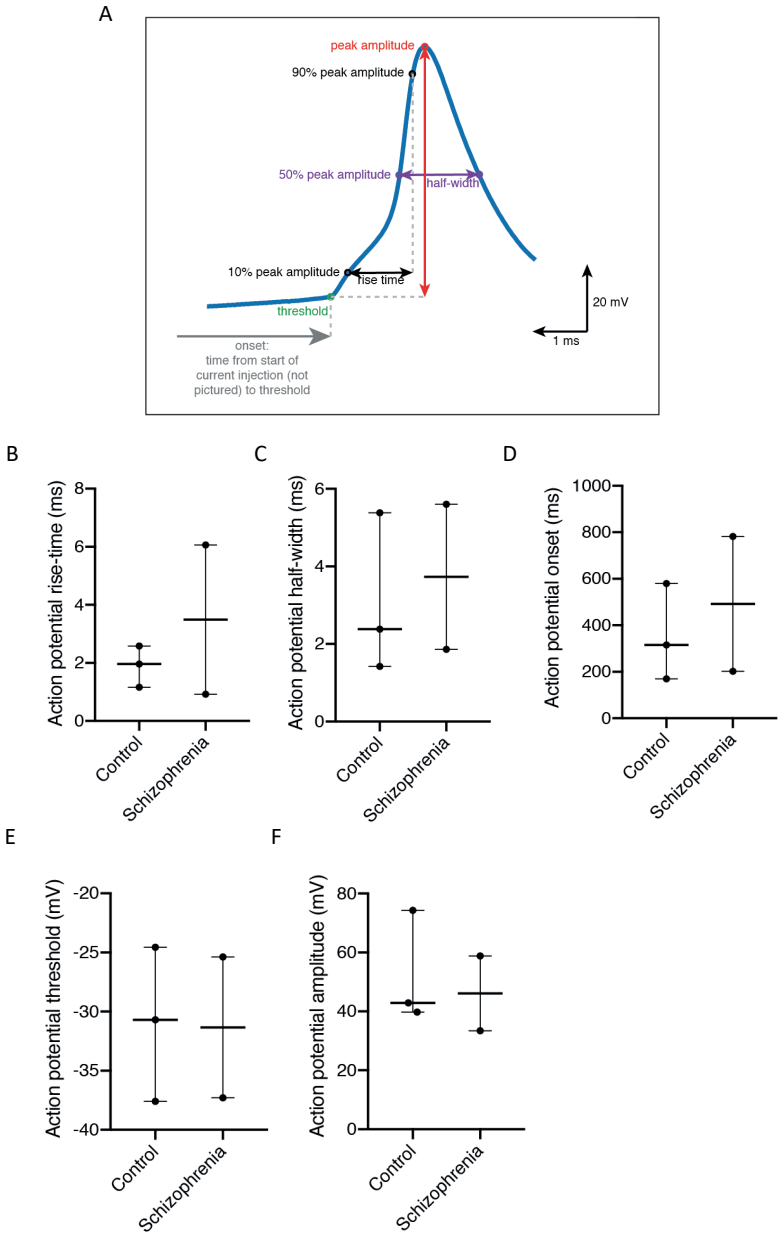


Figure 5: Electrophysiological properties of organoids **A)** Organoids (grown on Matrigel, which kept the cells from moving when approached with the recording pipette) were placed in the patch rig and secured in place using a modified harp that accounted for their smaller size relative to brain slices. **B)** Whole-cell recordings were made from cells (control: n = 25 cells; patient: n = 26 cells) using standard artificial cerebrospinal fluid and a potassium-gluconate internal solution. Image shows example of a recording pipette (rec) and recorded cell (white arrowhead)**. C)** Changes in membrane potential in

response to hyperpolarising and depolarising current steps were recorded from control (left) and patient-derived (right) cells. Three example recording traces are shown for each cell: the trace of the first current injection that was sufficient to elicit action potentials (rheobase current injection), 0 pA current injection and a hyperpolarising current injection equal to negative rheobase value. All cells were held at approximately -70mV and respective holding membrane potentials are noted to the left of each example trace. **D)** Passive membrane properties of control and patient-derived cells were not significantly **E)** Seven of 24 control cells and 11 of 25 patient-derived cells fired action potentials (APs) in response to positive current injection and displayed similar active with the exception of AP half-width, which was significantly faster in control cells Values are presented as either median, IQR (interquartile range) and p value from a Mann-Whitney test, or mean ± standard deviation with p value from a t test with Welch's correction

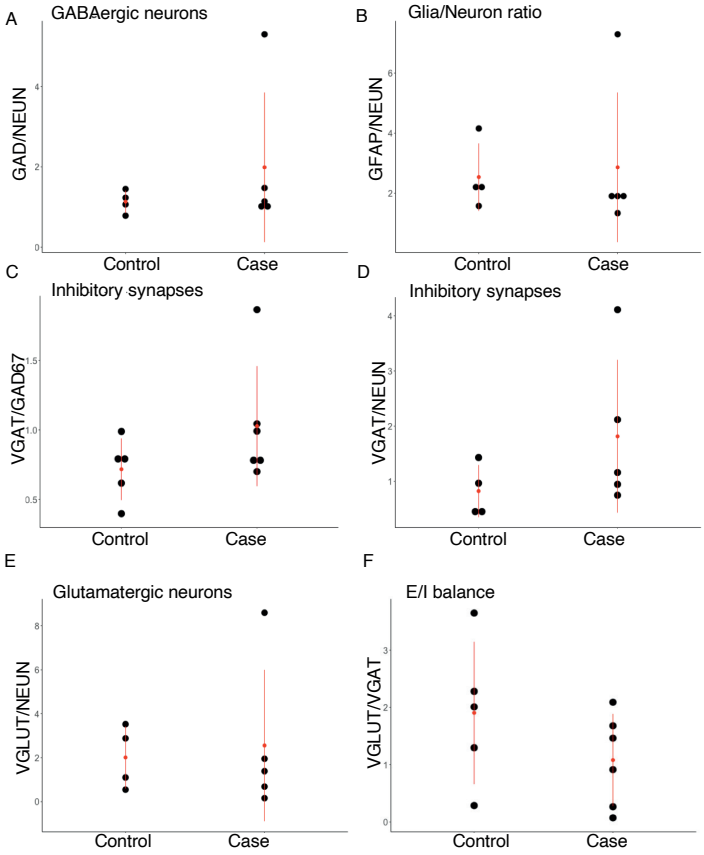


Figure 6: Quantification of synaptic markers (PCR). **A)** Ratio of interneurons (GAD65/67)/ all neurons (NEUN) **B)** Ratio of astrocytes (GFAP) and neurons (NEUN) **C)** Ratio of inhibitory synapses (VGAT) and inhibitory neurons (GAD 67) **D)** Ratio of inhibitory synapses (VGAT) and all neurons (NEUN) **E)** Ratio of excitatory synapses (VGLUT) and all neurons (NEUN) **F)** Ratio between excitatory synapses (VGLUT) and inhibitory synapses (VGAT). Each dot represents the ratio of one single individual. One measurement per individual was taken. There were no differences between SCZ patients and controls.

DISCUSSION

In the current report, we sought to find a model for the investigation of the additive effects of all brain cell types in SCZ-pathology. In addition, we aimed to generate a 3D model in order to model the complex spatial organization and self-assembly seen *in vivo.* Here we show preliminary results that our organoids generate cortical neurons, including glutamatergic (CAMKII-positive neurons) and GABA-ergic interneurons (GAD65/67-positive neurons). Moreover, we also show the presence of astrocytes and oligodendrocytes, confirming the generation of each cell type of interest. This suggests that this organoid model could indeed serve as a model for the investigation of such synergistic effects in SCZ, presuming we have functional neurons, astrocytes and oligodendrocytes. Patching CAMKII neurons further showed functional activity, however, the neurons revealed premature properties when compared to primary human neurons. This includes, higher resting membrane potential, higher membrane resistance accompanied by the need for strong depolarizing input currents for the generation of APs and relatively slow events. In addition, we showed that organoids form MBP-positive oligodendrocytes, that seem to myelinate, as they co-localized with NF200-positive axons. Despite that the figures showing the overlapping expression of MBP and NF200 look promising, myelination should be confirmed using 3D imaging to show that MBP is indeed wrapping completely around the axons. Overall, these results suggest that the current protocol can be used to model SCZ *in vitro* recapitulating the physiological 3D complexity of the human brain.

The current study was proceeded by investigation of phenotypic differences previously reported in SCZ. The presence of the different cell types allowed us to test potential skewness in differentiation tendencies. ICC for NF200, GFAP, SOX9 and OLIG2 did not indicate such skewness for any tested cell type. Our findings can therefore not confirm skewness towards GFAP-positive cells reported by Toyoshima et al. (2016). However, they are in line with another report (Szabo et al. 2021) who found no differences is skewness towards astrocytes and with our previous work (Chapter 5). Nor did we replicate the finding of Windrem et al. (2017) who showed a decrease in GFAP-positive cells in SCZ-derived differentiations. Similarly, we found no differences in differentiation tendencies towards oligodendrocytes based on OLIG2-positive cells. However, the variability between organoids from different cell lines was high, potentially undermining our ability to detect statistically significant results. Therefore, we do not replicate the differences in MBP surface area seen by De Vrij et al. (2018) and Windrem et al (2017) nor diminished OLIG2-positive cells shown by Windrem et al. Replication of the current experiments using larger sample sizes should clarify if the current lack of skewness is due to low statistical power caused by variability or if this phenotype is truly not present.

We further explored phenotypic differences between SCZ and control organoids by investigating the firing pattern of the neurons in our organoids. Patching of neurons expressing CFP revealed a difference in rise-time of APs in SCZ cases. A potential explanation for the delay of AP rise-time in the SCZ case driving the difference between the groups comes from his genetic background: this was the only case that carries the homozygous CACNA1C risk allele rs1006737. This gene encodes for the voltage gated L-type calcium channel $Ca_v1.2$. This allele has been shown by Yoshimitzu et al. (2015) to increase the density of L-type calcium currents. Moreover, Helton, Xu and Lipscombe (2005) showed that the $Ca_v1.2$ channel is relatively slow to open after stimulation and it takes relatively long to reach its peak after stimulation, which is consistent with the current results. Moreover, Plumbly et al. (2019) showed that the L-type calcium currents are crucial for normal network activity, and Paşca et al. (2011) showed that APs of neurons with dysfunctional $Ca_v1.2$ channels are indeed slower than APs generated by neurons with wild type $Ca_v1.2$ channels. Interestingly, the $Ca_v1.2$ channel is also expressed in astrocytes and the addition of siRNA against $Ca_v1.2$ mRNA significantly reduced calcium influx in astrocytes. Furthermore, this also prevented astrocyte reactivity induced by endotoxin liposaccharide *in vitro* (Cheli et al. 2016). As the risk allele rs1006737 is associated with higher mRNA transcripts and higher protein expression (Yoshimizu et al. 2015), this would could mean that astrocytes from homozygous carriers of this allele are more prone to reactivity, induced by liposaccharide, ATP, glutamate or increased extracellular potassium. Reactivity of astrocytes has been associated with SCZ, however, literature investigating this link is inconsistent (reviewed by Tarasov et al. 2020). Furthermore, the increase in current density reported by Yoshimitzu et al, is diagnosis independent as both homozygous controls and cases showed the increase calcium current density. In summary, the slower AP found here, are not representative for SCZ patients in general as it is not sufficient to develop SCZ nor it is limited to SCZ patients. Moreover, the effects of the risk allele in the homozygous patients can be attributed to neurons and to indirect effects mediated by astrocytes, since our cultures contained both cell types.

Our analysis did not reveal an E/I imbalance. However, at the time, we were unable to perform synaptic counts that we previously did in 2D culture to analyze E/I balance. The specificity of the synaptic antibodies was incompatible with the embedding in Matrigel, due to shortage of time to optimize the procedure. Using PCR analysis, we were not able to verify the results in Chapter 5. However, this does not imply that there is no difference in E/I imbalance in the current model, as we were only able to test mRNA levels, which does not allow the quantification of synapses. Therefore, potential synaptic alterations in the current model remain unclear. Further optimization should take place in order to obtain reliable synaptic visualization in the current cultures.

In the current chapter we show that the current model includes neurons, astrocytes and oligodendrocytes that self-assemble into a 3D sphere, where the different cell types interact creating a more complex and physiological model. Moreover, we show that neurons are functionally active and that oligodendrocytes express MBP and seem to myelinate the NF200 neuronal axons. However, the current complexity comes with its own limitations, such as higher variability and more specialized and complex analytical requirements compared to plain 2D models.

Moreover, since the emergence of more specialized and newer models to investigate oligodendrocytes and myelinations (Madhavan et al. 2018; Shaker et al. 2021) one should compare which method is most suitable to model the desired system. These models are more specialized towards the generation of oligodendrocytes, and could provide more measurements per organoids and potentially decreased variability.

MATERIALS AND METHODS

Subjects

7 patients with high polygenic risk score for schizophrenia (top 1%) were selected from the Swedish cohort. These individuals do not carry any SCZ associated CNVs. Furthermore, 6 healthy controls within the lowest 1% PRS were selected from the same cohort. None of these individuals carry any SCZ associated CNVs. In addition, we have selected 4 patients who did carry such a CNV. Three of them have a duplication in the 16p11.2 loci and one patient has a deletion at the 3q29 loci. The subjects are summarized in table 1.

Table 1: list of subjects included in the current study

Donors	Genotype	Sample size
Controls	Low PRS	4
SCZ cases	High PRS	6
SCZ cases	CNV	2

Cell culture

Fibroblasts were obtained from all patients, which were expanded in culture using fibroblast medium (DMEM-F12, 20% FBS, 1% NEAA, 1% pen/Strep, 50 µM β-Mercaptoethanol). Fibroblasts were reprogrammed as previously described (Hinz et al., 2018) and were brought in suspension (4x105 cells in 400 µl Gene Pulser Electroporation Buffer Reagent per well of a 6well plate; Biorad) together with 23.3 µg of each reprogramming plasmid (Plasdmid #27078, #27080, #27076, Addgene). Electroporation was done using a Gene

Pulser II (Biorad) in appropriate cuvettes. Pulser was set to 1.6 kV, 3 µF and 400 Ω and three pulses per reprogramming were applied. The cell suspension was transferred to a Geltrex precoated plate filled with fibroblast medium without antibiotics and supplemented with 10 µM ROCK-inhibitor (Y-27632), and the plate was incubated at 37 °C overnight. The next day, the medium was switched to normal fibroblast medium. When fibroblasts reached a confluence of 60-70%, the entire medium was replaced with TeSR™-E7™ (Stemcell) and was refreshed daily. Colonies formed were manually picked and transferred to Geltrex (Life Technologies) coated plates and kept in Essential 8 medium (Life Technologies), where after, EDTA passages were performed as previously described in (Holmes and Heine 2017).

iPSCs were differentiated towards neural precursor cells (NPCs) According to the protocol published by (Nadadhur et al. 2018). In brief, iPSCs were plated in a well of a 12 well plate in E8 medium with 10 µM ROCK-hinibitor, and half of the medium was refreshed the next day. The day after, 2/3 of the medium was replaced by Neural induction medium (Neural Maintenance Medium supplemented with 1 µM dorsomorphin (Trocis Bioscience) and 10 µM SB431 (SelleckChem). Cells were kept in this medium for 12 days, with daily refreshment of 2/3 of the medium.

After 12 days, neural rosettes were manually picked using a sterile insulin needle and were replaced in a PLO/mLAM [concentrations] coated well of a 12 well plate. Cells we left to attach overnight and received 50% medium change to NMM supplemented with 20ng/ml FGF2 (Peprotech) and 20ng/ml EGF (Peprotech) where after 50% of the medium was refreshed daily. NES cells were passaged at confluence using TrypLE. For the generation of Organoids in the current experiment, passage 4 NES cells were used. Furthermore, a modified version of the protocol by (Monzel et al. 2017) was used.

Cells were dissociated using TrypLE, and 9000 cells per were plated in one well of an anti-adhesive V-bottom 96 well plate (Sarsted) which was treated with Poly-hema in NMM lacking vitamin A, supplemented with 10 µM ROCK-inhibitor, 400 ng/ml hSHH, 3nM Chir-9902 and 50mg/ml Vitamine C (Sigma). Organoids were fed three times a week (50%). At day 10, half of the medium was switched to NMM lacking Vit A supplemented with 20ng/ml BDNF, 10 ng/ml GDNF, 10ng/ml IGF, 1 µM cAMP, 50mg/ml Vit C (Sigma) and 40ng/ml T3 (Sigma). Again, 50% of the medium was switched three days a week. At day 16, organoids were embedded in 30 µl growth factor reduced matrigel as described by Lancaster et al. Organoids were kept in culture until day 90. Two differentiations were performed for each subject.

Immunocytochemistry

Organoids were fixated in 4% paraformaldehyde, for 30 – 60 minutes, depending on their size. This was followed by three washing steps of 5 minutes each with PBS. Organoids were

transferred to a 30% sucrose solution and were incubated at 4°C for 2-3 days. This was followed by OCT embedding, first at 4°C for 30 minutes then at -80°C using dry ice and 2-methylbutane. Embedded organoids were kept at -80°C until cutting procedure. This was performed using a cryostat, set at -20°C (both chamber and blade). Sections of 12 and 30 µm were cut and mounted onto superfrost glass, then stored at -20°C until further processing.

Slides were left to acclimatize for 15 minutes, and were then washed in PBS 6 times (5minutes each time) on a shaker. Slices were blocked in PBS with 5% normal goat serum (gibco), 0.1% bovine serum albumin (Sigma), 0.3% Triton X-100 (Sigma) for 1 hour. Primary antibodies were dissolved in the same solution and incubated at RT for one hour and at 4°C O/N. After washing with PBS 3 times, secondary antibodies were dissolved in the same blocking buffer and incubated for 2 hours. Slides were washed 3 times in PBS and DAPI was added (in PBS for 5 minutes). Slides were again washed 3 times in PBS and embedded in Fluoromount™ (Sigma-Aldrich) and a coverglass was placed over the slices.

Primary ab: MAP2, NF200 (Sigma, 1:1000), MEIS1&2 (Santa Cruz), CTIP2 (Abcam, ab18465), synaptophysin

Secondaries: Alexa Fluor® 488 (ThermoFisher, 1:1000, goat anti mouse or rabbit), Alexa Fluor® 594 (ThermoFisher, 1:1000, goat anti mouse or rabbit).

SOX 9 (CellSignalling, 1:250, rabbit), GFAP (DAKO, 1:1000, rabbit and Sigma-Aldrich, 1:1000, mouse), S100b (ProteinTech, 1:1000, rabbit), Stainings to do: ID3 (Cell Signalling, 1:1000); MBP (Abcam 1:1000), Olig2 (Millipore 1:1000), MOG (Millipore, 1:500)

Columbus analysis

Organoid slices were stained as described above, and imaged using a Leica DM6000B fluorescent microscope. Pictures were taken systematically across the entire section of the organoid stating at the right top and moving towards the bottom left to avoid overlapping regions or a bias towards particular areas. Images were saved as PNG and uploaded to Columbus where they were analyzed in project mode. NF200 and GFAP staining were traced (using the find neurites function) and quantified. The same settings were used for the tracing of both markers. Since the slide does not allow tracing of neurites to particular cells, the sum of the neurite length was taken. To control for differences in density, the ratio of GFAP and NF200 was calculated for direct comparison between groups. Furthermore, Olig2 and Sox9 were quantified using the find nuclei function in Columbus and were normalized for cell density using Dapi counts (performed in the same way).

PCR analysis

Organoids were dissociated in Trizol and RNA was isolated using the classical chloroform and isopropanol method previously described in Chapter 5. complementary DNA was produced using Superscript IV and random hexameres.

Electrophysiology recordings

Organoids were placed in the patch rig and secures in place using a modified harp that accounted for their smaller size relative to brain slices. Cells were recorded using standard artificial cerebrospinal fluid (aCSF) and K-gluconate internal solution.

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug

treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380.e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen

FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J, White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical

region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity . Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684 . https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic

JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of "Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived

neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data

QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B,

Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

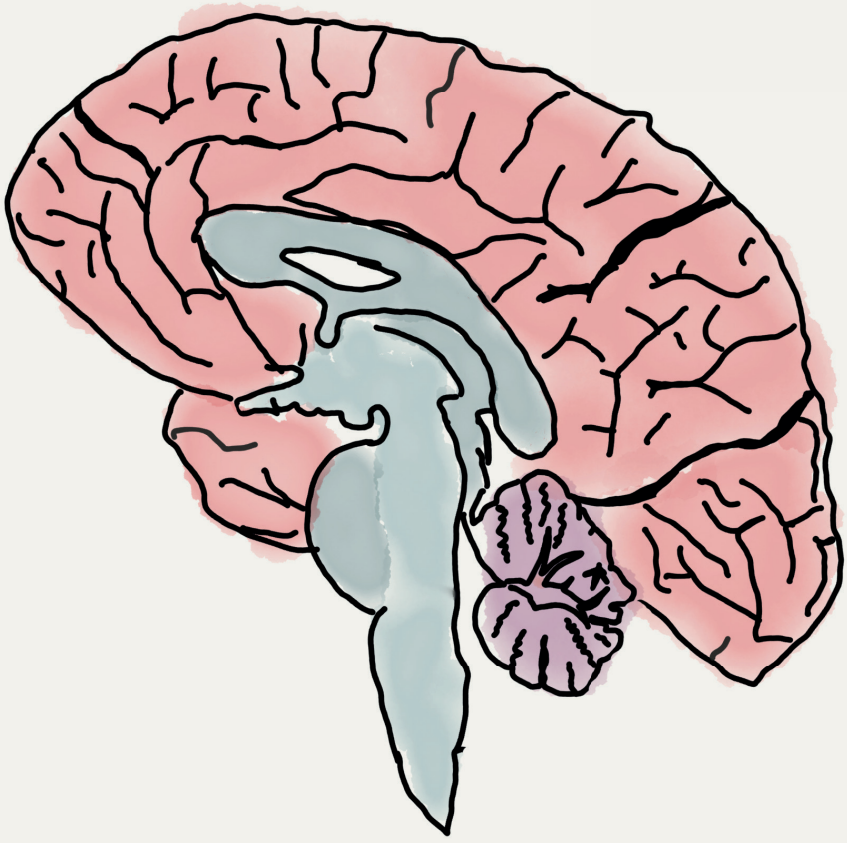
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Chapter 7

General discussion

Towards improved in vitro models for SCZ

The goal of the current thesis was to explore the role of astroglia in the pathology of SCZ using induced pluripotent stem cells. Therefore, central to this thesis is the comparison between cases and controls in chapters 5 and 6.

The current thesis began with determining the most ideal study population for SCZ, as SCZ is challenged by genetic complexity and iPSC studies by small sample sizes. In chapter 2 we decided to use polygenic risk scores (PRS) to select SCZ cases and controls. Specifically, we selected 10 patients with high PRS scores who did not carry any known SCZ-associated copy number variants (CNVs). The use of PRS leads to genetically more extreme groups of cases and controls which potentially increases statistical power by increasing the phenotypic differences between the two groups. The use of PRS also allowed us to investigate a potential (causal) phenotype in SCZ that was not influenced by *a priori* knowledge of potential causal single genes or pathways, employing an exploratory study set-up. For optimal conclusions about the causality of any phenotype detected, one should add high PRS controls and low PRS patients. As iPSC work is financially and labor intensive, it poses a strong limitation in sample size. Especially as the difference in cellular phenotype(s) between SCZ patients and healthy controls is expected to be small, a relatively large sample size is required. In order to increase the power of studies, we chose not to include high PRS controls and low PRS cases, which limits our interpretation of the phenotype. An additional limitation of the current PRS approach, is that the majority of SCZ-associated SNPs have not been identified yet. Therefore, the current PRS scoring is limited to the knowledge available at the time of scoring. This means that the scores will change after the revelation of newly identified causal SNPs. Yet, in favor of the current approach, we expect that the newly found SNPs will be either rarer or will have a smaller effect size than the current SNPs. This idea is based on the hypothesis that the heritability that is still 'missing' (as discussed in the introduction) has not been found yet due to lack of statistical power. As a consequence, we have likely selected the common variants with a relatively high effect size, which we believe will aid the detection of a phenotype in the current study.

In addition to the selection based on PRS, we also selected patients carrying specific, rare alleles of copy number variants (CNVs) that have been shown to have a relatively large effect on the risk for SCZ. This allows us to confirm any findings from the PRS cases/controls in a different genetic background, which would provide more widespread support for a causal role of our findings. We chose 6 cases carrying a 3q29 deletion and 5 cases carrying a 16p11.2 duplication based on the relatively high penetrance of these CNVs for SCZ and their relative low penetrance for other disorders (Kirov et al. 2014). The use of a (relatively) large sample size with three different genetic backgrounds lowers the chance of false positive

findings and enhances the chance of finding pathways that are truly associated with SCZ rather than with a specific genetic variant. The subjects included in the current thesis were obtained from the Karolinska Institutet in Sweden. All subjects participated in a previous GWAS projects of the PGC consortium (Ripke et al. 2013).

The next step in the optimization of our model was finding an appropriate reprogramming method to generate iPSCs for the selected cases and controls. Based on Chapter 3, we have chosen to reprogram the case and control fibroblasts episomally, as this does not lead to integration in the genome, and as it is a reliable but cost-efficient technique. Episomal reprogramming is widely used, and plasmids are freely available at Addgene at low cost. Still, in Chapter 3 we show that there is a difference between the episomal and lentiviral reprogramming when generating iPSCs from female donors: lentiviral reprogramming is able to induce X-chromosomal reactivation, while episomal reprogramming was not able to reactivate the inactive X-chromosome. These differences were confirmed by the mRNA expression of XIST. This could imply there is a difference between reprogramming efficiency between the two reprogramming methods. However, there were no differences in scores in the PluriTest between iPSC derived from lentivirus and episomal reprogramming, suggesting there was no difference in pluripotency between the two methods. Since the use of episomal reprogramming did not compromise pluripotency and since the episomal plasmids do not integrate and therefore cannot alter the genetic architecture of our iPSCs, this was in our case the favored method to derive iPSCs from study subjects.

In Chapter 4 we analyzed the variability of our iPSC-derived astrocytes (Tcw et al. 2017; Leferink et al. 2018; Szabo et al. 2021). More specifically, we investigated the variability throughout reprogramming and differentiation, giving insight in the origin of variability of our protocol. Three healthy donors were selected for reprogramming, where we included three iPSC-lines per donor. These lines were differentiated into astrocytes, one line per donor was differentiated three times in parallel. This set up allowed us to determine the source of the variability between our iPSC-derived astrocytes. We found that the magnitude of variability between clones remains stable throughout the culturing process. Moreover, we show that iPSCs and astrocytes derived from the same donor (clones) are more strongly correlated than iPSCs and astrocytes derived from different donors. This confirmed previous reports that genetic background is the main source of variability between samples. Moreover, there was no cell type specific protein set responsible for the variation between clones. Accumulation of mutations after reprogramming and long-term cultures did not explain the variability between clones in the current sample. Moreover, based on the correlations seen in this chapter, power simulations revealed that the use of multiple clones per donors would result in very limited gain in power, while the inclusion of more donors had much more impact on the statistical power to detect phenotypic differences between groups. Giving the high

financial costs and laborious nature of iPSC research, we chose to continue our SCZ study using a single clone per donor.

Human studies on mitochondrial alterations in SCZ

Using the optimized experimental set up described above, we generated iPSC-derived astrocytes using an established protocol (Tcw et al. 2017; Leferink et al. 2018; Szabo et al. 2021; Beekhuis-Hoekstra et al. 2021). When cultured in isolation, astrocytes did not reveal any differences between patients and controls for any of the tested phenotypes (several maturation factors and morphological properties were tested). On the contrary, transcriptome analysis revealed 10 DEGs between patients and controls, with *SLC25A18* as most statistically significantly down regulated in patients. In addition, SCZ astrocytes did induce differences in synapse formation in healthy neurons. More specifically, we show that there was a decrease in VGAT puncta in comparison to VGLUT2 or synaptophysin puncta. This suggests an impaired E/I balance as typically reported in SCZ. In support of a potential role of mitochondrial glutamate transporter 2 (GC2; encoded by *SLC25A18*), we show that the addition of exogenous glutamate according to the concentrations seen in a microdialysis report on SCZ patients (Eide and Stanisic 2010) indeed shifted the balance in VGLUT2 and VGAT puncta. In summary, our studies support central roles for astrocytes in SCZ pathology and suggest involvement of affected mitochondrial glutamate pathways. More specifically, we believe mitochondria could potentially act as an important point of conversion between the many pathways previously associated with SCZ (which is discussed in Chapter 5). An important limitation for this hypothesis is the lack of direct evidence for a mitochondrial deficit in the current thesis. For this reason, I will start the discussion by providing an overview of the overwhelming evidence revealing mitochondrial abnormalities in SCZ, which comes from a wide variety of study types (post mortem, genetics, patient studies, animal data and human *in vitro* models). With this I provide evidence for mitochondrial alterations in SCZ, that is currently lacking in this thesis, but might be a candidate for converging effects in SCZ. Even though there is much evidence in support of mitochondrial defects in SCZ, we are the first to show transcriptional and protein differences in GC2 expression in SCZ patients using an unbiased approach as discussed above. Furthermore, we would like to highlight the interplay between many of the different pathways associated with SCZ, which is summarized in Figure 1.

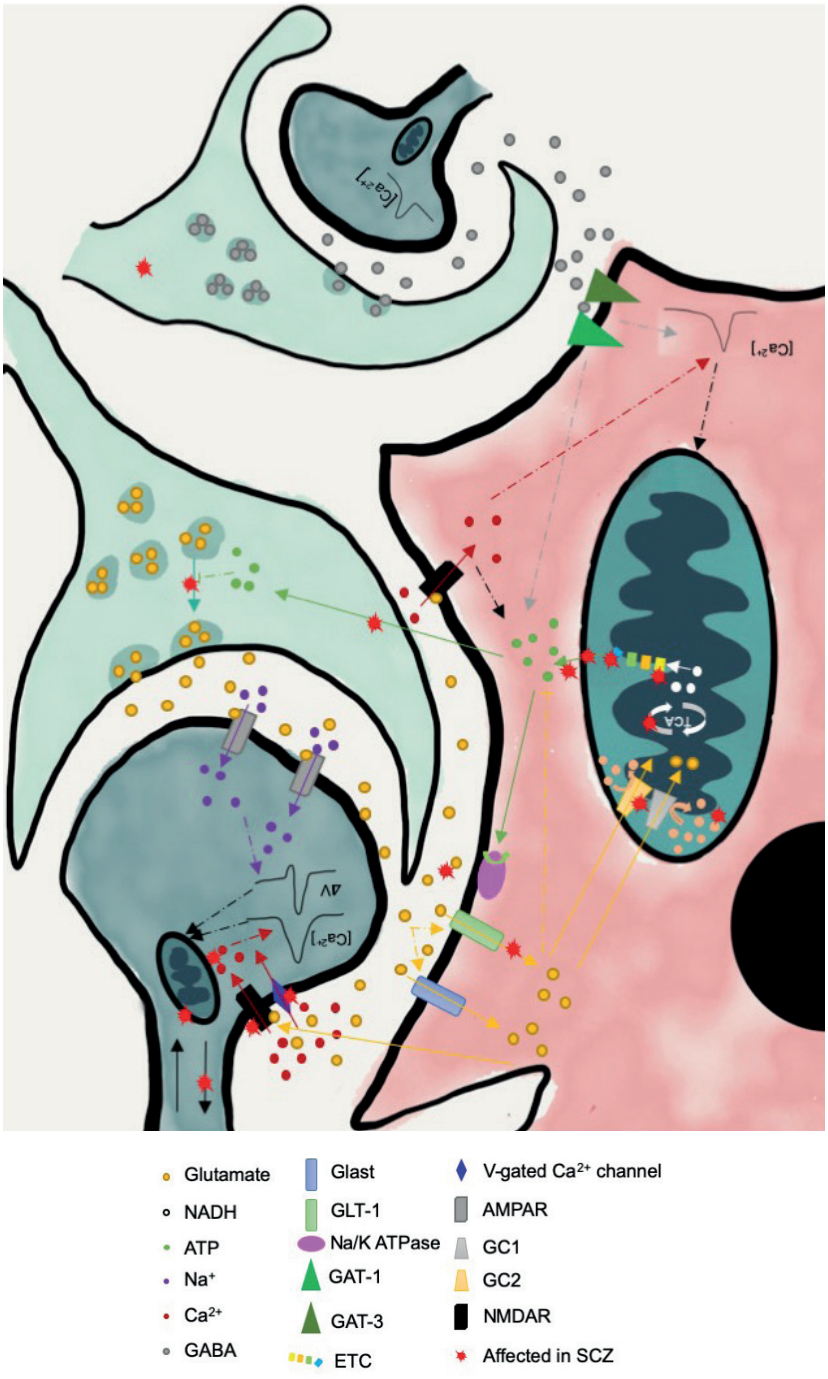


Figure 1: Overview of links between the different theories behind SCZ. This figure illustrates the connection between many SCZ-associated pathways.

Human studies on mitochondrial alterations in SCZ

We have identified GC2 as a potential candidate in SCZ-pathology, i.e. a protein that might act as an important point of conversion between the many pathways associated with SCZ (which is discussed in Chapter 5). An important limitation for this hypothesis is the lack of direct evidence for a mitochondrial deficit in the current thesis. For this reason, I will start the discussion by providing an overview of the overwhelming evidence revealing mitochondrial abnormalities in SCZ, which comes from a wide variety of study types (post mortem, genetics, patient studies, animal data and human *in vitro* models). I therefore hope to show that mitochondria in astrocytes form a strong candidate as a converging point of the various SCZ-associated hypotheses.

Firstly, there is post mortem evidence showing mitochondrial deficits in the brains of cases (Somerville et al. 2011; Arion et al. 2015). Such studies show decreased expression in genes encoding electron transport chain (ECT) complexes in SCZ (Maurer et al. 2001; Karry et al. 2004; Iwamoto et al. 2005). In accordance with a decreased energy production through the respiratory chain, increased ATP production through glycolysis has been reported (Regenold et al. 2012). These findings would have similar results as the decreased expression of GC2 seen in the current thesis, which would lead to a decreased supply of glutamate to the TCA-cycle. Oligodendrocytes from SCZ patients have shown decreased densities of mitochondria in the PFC and in the caudate nucleus, and the density of concentric lamellar bodies (which indicates the presence of damaged myelin fibers) was increased by 4,5-fold in the caudate nucleus of patients (Uranova et al. 2001), suggesting wide spread effects of the mitochondrial defects, which could also explain the wide variability of findings in SCZ. However, post mortem studies often donot provide insight into disease onset of neurodevelopmental disroders as it mainly consists of end-stage cases. Besides, they can be co-founded by many different factors (e.g. treatment) and do not always imply causality.

Secondly, there is also genetic evidence supporting this hypothesis. Nuclear encoded mitochondrial genes have associations with SCZ (Gonçalves et al. 2018), and enrichment analysis revealed a significant enrichment for mitochondrial genes in SCZ in the PGC2 GWAS (Schulmann et al. 2019), which also revealed 19 mitochondrial genes using Multi-Marker Analysis of GenoMic Annotation (MAGMA) analysis (Gonçalves et al. 2018). The widely known SCZ-associated 22q11.2 deletion harbors 6 mitochondrial genes. Furthermore, Shulman et al found that CTYB, a mitochondrial gene that encodes a protein in Complex III of the respiratory chain, is associated with SCZ and interacts with different nuclear genes previously associated with this disorder. Confirmation of the involvement of mitochondrial DNA in SCZ is the finding that there is a bias towards maternal inheritance for SCZ (Verge et al. 2012). Furthermore, many genes initially thought to be neuronal/synaptic can have additional, less well-known functions. One such example is DISC1. Splice variants of this

gene are expressed in mitochondria (Millar et al. 2005; Park et al. 2010). Moreover, DISC1 mutations have been shown to alter mitochondrion kinetics such as NADH dehydrogenase, ATP production and mitochondrial calcium dynamics (Park et al. 2010), which are all part of the tricarboxyliic acid cycle (TCA) cycle in the mitochodria. Kazuno et al also report two mitochondrial SNPs that alter mitochondrial pH and calcium dynamics, which have been previously associated with Parkinson, Alzheimer and bipolar disorder, confirming that mitochondrial function can indeed lead to brain specific effects and lead to human brain pathology, and might very well also underlie the pathology of SCZ.

Thirdly, there is also clinical data showing mitochondrial alterations between cases and controls. Comorbidity analysis revealed that patients with mitochondrial disorders can suffer from primary psychiatric symptoms, including psychosis and cognitive impairment (Anglin et al. 2012). In addition, there is a high degree of mitochondrial myopathy encephalopathy lactic acidosis (MELAS) among SCZ cases (Shao et al. 2008). Noteworthy, alterations to white matter abnormalities have been extensively reported in SCZ. Beyond the brain, there is primary tissue evidence coming from several studies reporting a reduction of mitochondrial DNA copy in blood or isolated leukocytes in SCZ (Li et al. 2015; Kumar et al. 2018). Furthermore, Kumar et al associated mitochondrial copy numbers in blood to psychosis severity but also after treatment with clozapine and risperidone, which raises the question if the differences in mitochondria are compensatory mechanisms that could potentially be beneficial. Indeed, others have shown that the function of mitochondrial ECT complex I was reduced in medicated patients compared to naïve patients (Casademont et al. 2007) and increased proton leakiness was found in blood-derived mitochondria of SCZ cases ((Bar-Yosef et al. 2020b) and discussed below). Moreover, treated patients show reduced pyruvate-malate oxidation (Casademont et al. 2007), which could indicate a dysfunction of the TCA-cycle, and would be in line with a reduction of ATP production by the respiratory chain. These results would imply that the patient-derived results discussed above are a consequence of the antipsychotic drugs taken by the patients, and could potentially be beneficial. However, atypical antipsychotics also inhibit complex I but to a lesser extent, and this extent of complex I inhibition of these drugs have been shown to correlate to their extrapyramidal side effects (Maurer & Möller, 1997), which are less prevalent in atypical antipsychotics compared to typical antipsychotics. On the other hand, mitochondrial defects were also shown in drug naïve patients (Li et al. 2015), suggesting that these findings are not merely reflecting drug effects. Moreover, the genetic association between mitochondrial genes and SCZ would also imply a causal role for mitochondrial dysfunctions. In addition, the copy number of mitochondrial DNA and mitochondrial function also decreases with age (Welle et al. 2003; Short et al. 2005). This included the production of ATP, and could be a potential explanation for the disease manifestation later in life. Together, these reports clearly

show the presence of mitochondrial deficits in SCZ and that this is not limited to the brain, however, the question remains if the mitochondrial effect are causal or beneficial to SCZ.

Lastly, in clinical trials, the chemicals and nutrients that are known to improve mitochondrial function have shown to give some amelioration in the cases, and further improvement when given as a supplement to conventional medication (summarized in Ni and Chung, 2020). This included improvement in Positive and negative Syndrome Scale (PANSS) scores, amelioration of extrapyramidal side effects, decrease of depressive and anxious feelings, lower risk of psychiatric morbidity, decreased loss of grey matter, improved working memory and protection against white matter loss in the fornix (Ni and Chung 2020). Besides, many reports have shown that antipsychotic treatment affects mitochondria. Interestingly, Bar-Yosef et al (2020) found that *in vitro* testing of mitochondrial alterations induced by antipsychotic treatment in blood-derived mitochondria of cases predicted the effectiveness of treatment *in vivo* in drug naïve patients. Moreover, only prolonged treatment was able to ameliorate mitochondrial dysfunction, which according to the authors, is in line with the duration-dependent mode of action of the treatment. The authors also show that there is a higher leakage of protons in mitochondria derived from cases, which was further enhanced by prolonged medication. The fact that antipsychotics act on mitochondrial pathways and that the amelioration of mitochondrial dysfunction in vitro could predict in vivo responsiveness to medication further forms strong evidence for a causal role of mitochondria in SCZ, with exception of the increase proton leakiness. Altogether, mitochondria have previously been hypothesized to be an intrinsic component of the SCZ etiology (Hjelm et al. 2015) and the recent findings discussed here underlie this hypothesis.

Model systems for SCZ present mitochondrial alterations

Traditional animal experiments support the idea of mitochondrial alterations in SCZ. For example, a large-scale mouse experiment involving three known CNVs associated with SCZ which showed a convergent effect of different SCZ-associated CNVs on metabolic pathways in the mouse cortex (Gordon et al. 2021). These researchers performed gene network analysis and report downregulation of metabolic genes in a variety of different genetic mouse models and a significant enrichment for genes downregulated in Autism Spectrum Disorders and SCZ postmortem brains. There is also an enrichment seen when this dataset was compared to SCZ GWAS results. Using hierarchical clustering on the network of gene found in their analysis, two cortical models were formed, one related to neuronal firing rate (inhibitory neurons in particular) that was of particular interest. Hub genes (defined as most correlated to their module) in their study were also mainly encoding mitochondrial protein located at the inner membrane of mitochondria, similar to GC2. Interestingly, two of the hub genes are involved in the TCA cycle and ATP synthesis (Idh3a and Atp5b), which both take place in mitochondria. A third hub gene (Bcat1) was involved in branched-chain amino acid

metabolism, which includes glutamate. Another interesting hub gene found in the module, that was consistently overexpressed in all CNVs, was Cend1, which is also found in the current RNAseq analysis to be downregulated in all patients (Chapter 5). This module mainly included genes that are specifically expressed in inhibitory cells, further strengthening the link between mitochondrial alterations, glutamate metabolism and alterations in inhibitory neurons/synapses. In line with the human data previously discussed, treatment of mouse models of SCZ (including Maternal Immune Activation models, ketamine- and amphetamine-induced models) with additives for mitochondrial support resulted in a reduction of PPI impairment, improvement of neurochemical imbalance, restoration of mitochondrial membrane potential and cortical connectivity, and reversal of cognitive deficits. This shows a direct association between mitochondrial deficits and SCZ-associated phenotypes in mice.

Animal studies show a direct link between mitochondrial alterations and neuronal activity. Local elevations in calcium result in the arrest of mitochondrial transport, particularly in nodes of Ranvier, and in spines. Inhibition of glutamate uptake increases mitochondrial mobility (Sheng and Cai, 2012). In line, Li et al showed that mitochondrial densities in dendrites are higher when neurons are depolarized, and lower when there is more inhibition. The activity in the synapses also affected the shape of mitochondria. Additionally, focal stimulation of neurons in the hippocampus can induce the invasion of mitochondria in enlarged synapses and this has been shown to be NMDAR dependent (Li et al. 2004). These authors thereby suggest that mitochondria are directly involved in synapse formation and development. They further confirm that depolarization inhibits the motility of mitochondria, and that this is mediated by NMDA-receptors but also by L-type voltage gated calcium channels. Additional confirmation from Rintoul et al (2003) also reveals that stimulation of neurons with glutamate at excitotoxic doses inhibits the movements of mitochondria and alters mitochondrial morphology favoring a more rounded shape, which has been associated with increased ROS and is calcium dependent (Ahmad et al. 2013). These results show that mitochondrial alterations, glutamate concentrations, NMDA receptors and calcium signaling are directly linked and interestingly all associated with SCZ. Noteworthy, the link between the mitochondria and network function is bidirectional, as further elaborated below.

Also human primary cell culture provides experimental evidence for a mitochondrial dysfunction in SCZ, as differences in mitochondrial proteins such as SLC25A1 and SLC25A4 (both expressed in the mitochondrial inner membrane) have been shown in fibroblast of 22q11.2 deletion syndrome patients (Gokhale et al. 2019). These proteins interact with other SLC25A family members, are involved in the respiratory chain and are crucial for synapse development and maintenance of their mitochondrial pool. Mitochondrial deficits have also previously been associated with SCZ in iPSC-derived cells. Robicsek et al (2013) have reported that the mitochondrial distribution in SCZ patient iPSC-derived neuroepithelial stem

(NES) cells was altered and that their membrane potential was lower. The latter is particularly in line with our findings, as the proton gradient across the inner membrane is of crucial importance for the mitochondrial membrane potential. More evidence from the association with proton leakiness (and thus mitochondrial membrane potential) in SCZ comes from studies associating variants located in genes encoding uncoupling proteins (UPCs) to the disorder (Mouaffak et al. 2011). A more recent study found that neurons derived from SCZ patients with a 22q11.2 deletion, show a decrease in mitochondrial proteins MT-ND1, cytochrome B and COX1 involved in the electron transport chain in SCZ cases (Li et al. 2019). This was accompanied by a decrease in ATP production due to decreased function of the respiratory chain complexes I and IV. These studies show that iPSC models can mimic mitochondrial defects in SCZ, at least in neural progenitor populations. We are the first to suggest mitochondrial alterations in astrocytes, mediating SCZ-like deficits in neuronal networks.

Mitochondrial alterations in SCZ astrocytes, glutamate & hyperexcitability

In the current thesis we show that SCZ astrocytes can impact network formation, and induce an imbalance between excitatory VGLUT-positive synapses and inhibitory VGAT-positive synapses. It is suggested that this phenotype is induced by mitochondrial alterations in SCZ astrocytes, as the expression of mitochondrial glutamate carrier GC2 is altered in these cells. As discussed in the general introduction, there are several reports showing glutamatergic alterations in SCZ astrocytes, and given the role of GC2, mitochondrial alterations and increased glutamate levels, these observations may be connected in SCZ. I will discuss how mitochondria are key players in glutamate clearance, which takes place in astrocytes and strongly affects neuronal network functioning. More specifically, I will follow with a discussion into how mitochondrial alterations in astrocytes directly affects inhibitory neurons through the production and secretion of ATP.

Role of GC2 in regulating glutamate homeostasis

The kinetics of GC2 (encoded by *SLC25A18*) and of its homolog GC1 (encoded by *SLC25A22*) differ, as the binding affinity for glutamate is higher for GC2 than GC1, and the pumping speed is higher for GC1 than GC2 (Fiermonte et al. 2002). This implies that GC2 is the main responsible carrier for the clearance of glutamate in the cytosol of astrocytes under conditions where glutamate concentrations in the cytosol are relatively low. When cytosolic glutamate levels rise, GC1 rapidly pumps glutamate across the inner mitochondrial membrane. Under basal conditions, 30% of glutamine is oxidized by the TCA-cycle, which increases when glutamate concentration increases. This would require additional pumping by GC1 and GC2. Azarias et al. (2011) showed that glutamate transport by astrocytes induced a decrease in the mitochondrial pH, which was accompanied by a decrease in mitochondrial reactive oxygen species (ROS) production. This indicates a decrease in

mitochondrial respiration in SCZ as hypothesized in the current study and previous studies, as discussed above (Ben-Shachar 2017; Li et al. 2019). In addition, it is in line with the normal functioning of GC1 and GC2, which transport glutamate in exchange for a proton. Goubert et al. (2017) showed that downregulation of GC1 (by 80%) results in the accumulation of glutamate in the cytosol of astrocytes and reduced ATP production. Moreover, mutations in the SLC25A22 gene have been reported to induce epilepsy. This provided the first evidence that mitochondrial defects can lead to hyperexcitability, which is mediated through glutamate accumulation in astrocytes.

Astrocytes and glutamate excitotoxicity

The uptake of glutamate by astrocytes can significantly impact neuronal network functioning, as the glutamate uptake regulates glutamate spillover that activates glutamatergic metabotropic receptors such as NMDAR. This has been determined by altering the expression of glutamate transporters in astrocytes (Huang et al. 2004). Generally, glutamate transporters maintain a low concentration of glutamate near receptors to avoid chronic receptor activation and desensitization, which ensures the appropriate signal-to-noise ratio (Robinson et al. 2004). The spillover shapes the excitatory postsynaptic currents (Murphy-Royal et al. 2015) and therefore also impacts synaptic plasticity. Vice versa, astrocyte function is directed by synaptic input. For example LTP in hippocampal neurons can increase the expression of GLT-1 and GLAST in astrocytes, resulting in an increased glutamate uptake abilities (Pita-Almenar et al. 2006; Pita-Almenar et al. 2012). In accordance, sustained stimulation of the same neuronal pathways leads to long-term increase in glutamate secretions of astrocytes, which stimulates neuronal NMDAR activation and boosts synaptic plasticity (Genoud et al. 2006; Pirttimaki et al. 2011). This shows that neuronal activity regulates astrocytic pathways involved in glutamate metabolism.

Mitochondrial electron transport chain and glutamate excitotoxicity

Glutamate metabolism is probably (partially) mediated by mitochondria: GLT-1 co-immunoprecipitated with 25 mitochondrial proteins (Genda et al. 2011), which included GC1. At least two mitochondrial proteins and two Na/K ATPase subunits interact with GLAST and there is a substantial co-localization of GLT-1 and mitochondria (70%) in astrocytic processes suggesting a close association between glutamate clearance and ATP metabolism. This is seen to a lesser extent with GLAST (50%). Moreover, astrocytic ATP release is essential for network functioning: it has previously been shown that depending on the frequency and duration of interneuron firing, astrocytes either release glutamate to strengthen excitatory activity or they release ATP/adenosine to reduce excitatory transmission (Perea et al. 2016; Covelo and Araque 2018). This shows that ATP release and glutamate metabolism are tightly linked, and that mitochondrial ECT activity can have a major impact in network functioning

through ATP release of astrocytes. Interestingly, all these pathways have been previously associated with SCZ.

Astrocytes also take up GABA through GAT-1 and 3. This uptake leads to increased calcium concentrations (Doengi et al. 2009) thereby stimulating the release of ATP, which further inhibits glutamatergic synaptic transmission (Boddum et al. 2016). Abnormal calcium signaling alters GAT expression and GABA uptake leading to behavioural deficits. Interestingly, ATP production was affected in SLC25A4 null human cells and hemideficiency in the drosophila SLC25A1 and SLC25A4 ortholog lead to abnormal network formations, oscillatory behaviour of neurons and altered sleep patterns in the flies. Human astrocytic domains can cover between 270 000 and 2 million synapses (Mederos et al. 2018). By their release of ATP/adenosine and glutamate, astrocytes regulate cortical oscillatory patterns, such as slow oscillations during sleep (Fellin et al. 2004; Poskanzer and Yuste 2016; Clasadonte et al. 2017). This shows that astrocytes can act as appoint of integration for large network areas, directly influencing behavior through their influence on oscillatory patterns. This further links SCZ-associated pathways GABA, to glutamate, mitochondrial ECT activity and ATP release by astrocytes and altered oscillatory behavior.

As astrocytes show high heterogeneity and astrocyte subtypes are likely coupled through different tight junctions via which they exchange potassium, glutamate, ATP, cAMP. These networks are crucial for many higher order brain functions including cognition and behavior (Charvériat et al. 2017), in which astrocytes might play a larger role than so far assumed. Furthermore, the action of the NMDA receptor has generally been more associated with neurons, but they are also present in the astrocytes, where they act through influx independent manor (Montes de Oca Balderas 2018). Potentially, the psychotic effects of NMDARs could also be mediated by the astrocytes, rather than by neurons. NMDA stimulation of astrocytes induces ATP release, which is dependent on the electron transport chain. The following mechanism is suggested by Letelier and colleagues (Letellier et al. 2016) : In the tripartite synapse, the presynaptic terminal of a glutamatergic neuron releases glutamate, which will not only bind to receptors on the postsynaptic terminal, but also on NMDARs of astrocytes. This will elicit a calcium wave within the astrocyte that in turn leads to the release of ATP, which acts as an inhibitory transmitter on excitatory synapses. In summary, astrocytes orchestrate neuronal network functioning through extrasynaptic NMDAR activation by means of glutamate clearance, which is in turn dependent on mitochondria. Consequently, glutamate metabolism affects the ECT and ATP production in astrocytic mitochondria, which again has been shown to affect glutamate clearance and directly impacts neuronal network. In addition, activation of the NMDAR in astrocytes also directly leads to ECT chain dependent ATP signaling that aids in maintenance of network behaviour. And again, alterations in glutamate clearance (as

seen in GC1 mutations), ATP production or release results in hyperexcitability, are all broadly associated with SCZ.

Changed astrocyte glutamate metabolism in SCZ: cause or consequence

To put the current results in perspective were we find GC2 protein overexpression, we can hypothesize that GC2 plays a crucial role in SCZ pathology through glutamate homeostasis. Potentially, GC2 overexpression can be a consequence of increased glutamate release by neurons, as rising glutamate levels induce a shift towards its oxidative metabolism as previously discussed. This would imply that GC2 does not have a causal role in SCZ, but rather that the increase in protein levels is a compensatory mechanism set in motion to process the excess glutamate from the extracellular space. However, in our cultures, GC2 overexpression was seen in astrocytes that were not in contact with neurons, suggesting that the overexpression is not a consequence of altered neuronal network. Alternatively, the overexpression of GC2 could be due to an accumulation of glutamate in the astrocyte cytoplasm as a failure of glutamate metabolism by glutamate synthase. This would lead to a reduction in glutamate uptake as GLT-1 and GLAST are gradient dependent. This is supported by the findings of Szabo et al who showed that iPSC-derived astrocytes from patients have decreased capacity of glutamate uptake compared to iPSC-derived astrocytes from controls (Szabo et al. 2021). In addition, patient studies have shown increased concentrations of glutamate in cases, and the involvement of astrocyte glutamate uptake has been previously hypothesized (Marder and Cannon 2019). Yet another explanation would be that the decreased ECT functioning of the SCZ mitochondria leads to GC2 overexpression in order to recruit more glutamate to feed the TCA cycle. The glutamate pumped into the mitochondria is the only source of a-ketogluratate for the TCA cycle (Satrústegui et al. 2007). Therefore, the production of ATP is dependent on the pumping capacities of these transporters. This is supported by the numerous findings as discussed above, showing decreased ATP production in SCZ cases. This would again suggest that the overexpression of GC2 is compensatory to either glutamate accumulation or to a reduction of ATP production.

Indirect evidence for the hypothesis that SLC25A18 might affect glutamate uptake comes from a study showing that the glutamate uptake in the anterior cingulate cortex (ACC) differs from the sensory cortex under high frequency stimulation. This study shows that under high frequency stimulation, astrocytes in ACC increase their glutamate uptake, while in the barrel cortex the astrocytes decrease glutamate uptake upon the same stimuli (Romanos et al. 2019). This implies a strict regulation of glutamate in the ACC, and alterations thereof could underlie psychotic symptoms. Given the preferential expression of GC2 in the ACC, GC2 might take part in the fast glutamate clearance in this region. Moreover, the finding that duplications in SLC25A18 in Russian genetic isolate seems to be associated with SCZ, would argue for a causal role of GC2. It thus remains unknown if the overexpression in

GC2 is causal or beneficial for SCZ patients. It is clear, however, that mitochondria are associated with several pathways that are in turn consistently associated with SCZ (e.g. glutamate homeostasis, NMDAR hypofunction, calcium signaling and dysconnectivity). More importantly, astrocytes seem to be the place where these pathways converge, and they have been shown to alter network connectivity. Therefore, we can conclude that astrocytes are of crucial importance in SCZ pathology.

Mitochondrial alterations in SCZ & white matter abnormalities

Our studies do not include any investigation on potential mitochondrial dysfunctions in other neural cell types in the SCZ brain, or a role for astrocytes in other SCZ-associated phenotypes, e.g. white matter abnormalities. MRI studies have shown alteration in white matter of SCZ patients (Xiao et al. 2018; de Vrij et al. 2019; Cetin-Karayumak et al. 2020). Furthermore, several studies have reported abnormalities in oligodendrocytes and white matter abnormalities in SCZ, which is also confirmed by iPSC studies (Windrem et al. 2017; de Vrij et al. 2019). Interestingly, patients with white matter abnormalities (leukoencephalopathies) often present psychotic episodes, and white matter abnormalities have been widely recognized as a feature of mitochondrial diseases (Wong 2012). The following mitochondrial diseases result in white matter abnormalities (i.e. present as leukoencepholopathies): mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), Kearns–Sayre's syndrome (KSS), mitochondrial neurogastrointestinal encephalopathy (MNGIE) and Alpers syndrome. Comparably, several leukoencepholopathies are caused by mutations in mitochondrial genes, including Leigh's disease, leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) or leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL), optic atrophy (DOA) (mutations in OPA1) and Charcot–Marie–Tooth Types 2A and 6A (mutations in mitofusin 2) (Morató et al. 2014).

Cellular studies support important roles for mitochondria in white matter development and maintenance. Disruption of mitochondrial function in oligodendrocytes negatively affects their differentiation, viability and their capacity to form myelin sheets (Morató et al. 2014). Moreover, astrocytes can play crucial roles in white matter pathology as illustrated by Alexander's disease (Molofsky et al. 2012). Interestingly, essential roles of astrocytes rely on mitochondrial functioning: modulation of neurotransmission and energy supply (Morató et al. 2014). Glutamate clearance and metabolism, ATP and lactate production (astrocytes also supply neurons with lactate) all rely on mitochondria. Interfering with these pathways (aralar mice) and AGC1 deletions do not only affect network functioning, but also lead to hypomyelination. In addition, mitochondria have been shown to play crucial roles in microglia, where they seem to be involved in the production of pro-inflammatory mediators, which means they could partially be responsible for neuroinflammation (another pathways that has been linked to SCZ) (Joshi et al. 2019). Moreover, GC2 expression increases upon

inflammatory stimulation in macrophages (Hans et al. 2019), potentially linking GC2 to (neuro)inflammation. Future studies must show a central role of mitochondria in other SCZ brain phenotypes. Considering the white matter consists of several neuronal and glial cell structures, the brain organoid cultures showing myelin structures that have been evolving in recent years, might present promising modeling systems to tackle these follow-up questions.

Mitochondrial alterations in SCZ & the dopamine hypothesis

Mitochondrial dysfunction could also underlie the dopamine hypothesis as dopamine has been shown to inhibit mitochondrial respiration (Ben-Shachar 2017). The byproducts of dopamine can create oxidative stress and Ben-Shachar showed that dopamine actively targets the mitochondria. The outer membrane of the mitochondria express monoamine oxidase, and mitochondria take up dopamine, where dopamine can interact with complex I of the respiratory chain (Ben-Shachar and Laifenfeld 2004; Ben-Shachar 2020), and leads to decreased ATP production. Moreover, as discussed, several antipsychotic drugs are also seen to inhibit this complex I (Balijepalli et al. 1999; Balijepalli et al. 2001) although it is unclear if this happens directly or through indirect mechanisms. Moreover, this could be beneficial, suggesting that decreased complex I activity is a compensatory mechanisms. Ben-Shachar also suggests that the decrease in mitochondrial respiration is possibly a compensation of increased dopamine levels in SCZ. However, in the current experiments, there is no dopamine present suggesting that the increase of GC2 is not a compensatory mechanism for increased dopaminergic levels specifically. Future studies will tell whether glial dysfunctions in SCZ play a role in the dopamine hypothesis.

General conclusions and future research

In the current study we provide direct evidence that SCZ astrocytes alter neuronal network with an underlying role for glutamate metabolism and mitochondria. Moreover, our results might clarify the many different findings in SCZ etiology. I would like to pose mitochondria as a convergent mechanism that links many different findings in SCZ, ranging from the classic hypotheses such as dopamine abnormalities and dysconnectivity to more recent findings in white matter abnormalities and inflammation. Furthermore, I would like to highlight the role for astrocytes in the altered neuronal network in SCZ.

Important strengths of the current study are the relatively large sample size, our genetically stratified sampling method, which does not require a priori knowledge of causal pathways. The latter allowed us to perform an unbiased exploration of potential causal pathways in SCZ astrocytes. Moreover, this sampling strategy also gives us higher statistical power to detect differences between the effects control and SCZ astrocytes have on neuronal network formation.

Our study also poses some limitations, such as the lack of direct evidence for glutamate metabolism and mitochondrial dysfunction. Moreover, the commercially available medium used to culture our astrocytes contains fetal bovine serum (FBS), which has been shown to alter astrocyte morphology and transcriptome towards type A1 (reactive astrocyte subtype). Despite the overwhelming evidence for their role in SCZ, future research should elaborate on the link between mitochondrial alterations, glutamate metabolism and dysconnectivity. The current work could be elaborated by investigating the glutamate uptake by our SCZ and control astrocytes through high pressure liquid chromatography or glutamate-sensing fluorescent reporter (iGluSnFR). Ideally, these results should be coupled to mitochondrial metabolism, which can be performed by tetramethylrhodamine methyl ester perchlorate (TMRM, or other dyes to microscopically visualize the membrane potential of mitochondria), or by analyzing the oxygen consumption rates (e.g. with the Agilent Seahorse XF). Finding alterations in glutamate uptake and/or mitochondrial metabolism would provide further evidence for the current hypothesis. Moreover, experimentally manipulating mitochondrial respiration and investigating glutamate uptake, should provide evidence for a direct role of the mitochondria in glutamate uptake. In addition, repeating our co-culture experiments with healthy neurons should help to understand if the shift towards VGLUT-positive synapses seen in the current thesis could indeed be a result of mitochondrial abnormalities. In accordance, rescuing the any presented mitochondrial phenotype should lead to normal neuronal network formation. Moreover, electrophysiological measurements, particularly microelectrode arrays, could reveal the functional impact of the current synaptic imbalance on a network. In addition, the rescue of this phenotype could be tested by the application of antipsychotic drugs (preferably drugs that have been shown to alleviate symptoms in the patients from whom the iPSCs have been derived, if that information is available). The GC2 expression and any potential mitochondrial defects can also be reassessed after drug treatment, which should shed light onto the causal pathways of SCZ.

After revealing the cascade of events that lead to the E/I-imbalance seen in the Chapter 5, this research could be taken further by determining the effects of mitochondrial alterations, glutamate uptake and disinhibition to a more complex models such as the organoid model in Chapter 6. This model will allow the investigation of any phenotypes in oligodendrocytes and on myelination.

Moreover, the vast majority of the findings of astrocytic involvement in network formation and tripartite synapse, are derived from animal studies. Additional research needs to be performed on human (iPSC-derived) astrocytes, as they have been shown to differ from their rodent counterpart in many different ways. Most importantly, not all human astrocyte subtypes are present in rodents (interlaminar and varicose astrocytes (Colombo and Reisin 2004; Oberheim et al. 2006)). Additionally, human protoplasmic astrocytes do not obey

the domain organization seen in rodents, and they contact 20 times more synapses than rodent protoplasmic cells (Oberheim et al. 2006). Additional differences include membrane capacitance, speed of calcium waves and gene expression (Oberheim et al. 2009; Oberheim et al. 2012; Vasile et al. 2017). Strikingly, only 30% of genes enriched in human astrocytes have a mouse ortholog (Zhang et al. 2014). The latter forms a major limitation in SCZ considering the polygenic nature of this disorder. For future research, it would be interesting to explore the representation of all astrocyte subtypes in out models and how each subtype can influence network behavior.

REFERENCES

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D₂ receptors by dopamine in schizophrenia. Proc Natl Acad Sci 97:8104 LP – 8109 . https://doi.org/10.1073/pnas.97.14.8104

Adityanjee, Aderibigbe YA, Theodoridis D, Vieweg WVR (1999) Dementia praecox to schizophrenia: The first 100 years. Psychiatry Clin Neurosci 53:437–448 . https://doi.org/10.1046/j.1440-1819.1999.00584.x

Ahmad T, Aggarwal K, Pattnaik B, Mukherjee S, Sethi T, Tiwari BK, Kumar M, Micheal A, Mabalirajan U, Ghosh B, Sinha Roy S, Agrawal A (2013) Computational classification of mitochondrial shapes reflects stress and redox state. Cell Death Dis 4:e461 . https://doi.org/10.1038/cddis.2012.213

Akkouh IA, Hughes T, Steen VM, Glover JC, Andreassen OA, Djurovic S, Szabo A (2021) Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia. Brain Behav Immun 94:235–244 . https://doi.org/10.1016/j.bbi.2021.01.037

Akkouh IA, Ueland T, Hansson L, Inderhaug E, Hughes T, Steen NE, Aukrust P, Andreassen OA, Szabo A, Djurovic S (2020) Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav Immun 87:634–644 . https://doi.org/10.1016/j.bbi.2020.02.008

Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI (2012) The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. J Clin Psychiatry 73:506—512 . https://doi.org/10.4088/jcp.11r07237

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang X-J, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. J Neurosci 35:267–286 . https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Arion D, Corradi JP, Tang S, Datta D, Boothe F, He A, Cacace AM, Zaczek R, Albright CF, Tseng G, Lewis DA (2015) Distinctive transcriptome alterations of prefrontal pyramidal neurons in schizophrenia and schizoaffective disorder. Mol Psychiatry 20:1397–1405 . https://doi.org/10.1038/mp.2014.171

Azarias G, Perreten H, Lengacher S, Poburko D, Demaurex N, Magistretti PJ, Chatton J-Y (2011) Glutamate Transport Decreases Mitochondrial pH and Modulates Oxidative Metabolism in Astrocytes. J Neurosci 31:3550 LP – 3559 . https://doi.org/10.1523/JNEUROSCI.4378-10.2011

Balijepalli S, Boyd MR, Ravindranath V (1999) Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropharmacology 38:567–577 . https://doi.org/10.1016/s0028-3908(98)00215-9

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435 . https://doi.org/10.1016/s0197-0186(00)00108-x

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020a) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Bar-Yosef T, Hussein W, Yitzhaki O, Damri O, Givon L, Marom C, Gurman V, Levine J, Bersudsky Y, Agam G, Ben-Shachar D (2020b) Mitochondrial function parameters as a tool for tailored drug treatment of an individual with psychosis: a proof of concept study. Sci Rep 10:12258 . https://doi.org/10.1038/s41598-020-69207-4

Beekhuis-Hoekstra SD, Watanabe K, Werme J, de Leeuw CA, Paliukhovich I, Li KW, Koopmans F, Smit AB, Posthuma D, Heine VM (2021) Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res 56:102512 . https://doi.org/10.1016/j.scr.2021.102512

Ben-Shachar D (2017) Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. Schizophr Res 187:3–10 . https://doi.org/10.1016/j.schres.2016.10.022

Ben-Shachar D (2020) The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia. J Neural Transm 127:159–168 . https://doi.org/10.1007/s00702-019-02120-x

Ben-Shachar D, Laifenfeld D (2004) Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 59:273–296 . https://doi.org/10.1016/S0074-7742(04)59011-6

Bernstein H-G, Steiner J, Guest PC, Dobrowolny H, Bogerts B (2015) Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr Res 161:4–18 . https://doi.org/10.1016/j.schres.2014.03.035

Boddum K, Jensen TP, Magloire V, Kristiansen U, Rusakov DA, Pavlov I, Walker MC (2016) Astrocytic GABA transporter activity modulates excitatory neurotransmission. Nat Commun 7:13572 . https://doi.org/10.1038/ncomms13572

Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473:221–225 . https://doi.org/10.1038/nature09915

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, Harkavy-Friedman J, Gorman JM, Malaspina D, Susser ES (2002) Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533 . https://doi.org/10.1176/appi.ajp.159.9.1528

Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH (2001) Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry 58:1032–1037 . https://doi.org/10.1001/archpsyc.58.11.1032

Bulayeva K, Bulayev O, Glatt S (2016) Genomic architecture of schizophrenia across diverse genetic isolates: A study of dagestan populations

Burkhardt C, Kelly JP, Lim Y-H, Filley CM, Parker Jr WD (1993) Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 33:512–517 . https://doi.org/10.1002/ana.410330516

Canitano R, Pallagrosi M (2017) Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories. Front psychiatry 8:69 . https://doi.org/10.3389/fpsyt.2017.00069

Cannon TD (2015) How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci 19:744–756 . https://doi.org/10.1016/j.tics.2015.09.009

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17

Casademont J, Garrabou G, Mir?? ??scar, L??pez S, Pons A, Bernardo M, Cardellach F (2007) Neuroleptic Treatment Effect on Mitochondrial Electron Transport Chain. J Clin Psychopharmacol 27:284–288 . https://doi.org/10.1097/JCP.0b013e318054753e

Casas BS, Vitória G, do Costa MN, Madeiro da Costa R, Trindade P, Maciel R, Navarrete N, Rehen SK, Palma V (2018) hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl Psychiatry 8:48 . https://doi.org/10.1038/s41398-018-0095-9

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, James A, Voineskos A, Buchanan RW, Szeszko PR, Malhotra AK, Hegde

R, McCarley R, Keshavan M, Shenton M, Rathi Y, Kubicki M (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208–3219 . https://doi.org/10.1038/s41380-019-0509-y

Charvériat M, Naus CC, Leybaert L, Sáez JC, Giaume C (2017) Connexin-Dependent Neuroglial Networking as a New Therapeutic Target. Front Cell Neurosci 11: . https://doi.org/10.3389/fncel.2017.00174

Cheli VT, Santiago González DA, Smith J, Spreuer V, Murphy GG, Paez PM (2016) L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia 64:1396–1415 . https://doi.org/10.1002/glia.23013

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron 95:1365-1380.e5 . https://doi.org/https://doi.org/10.1016/j.neuron.2017.08.022

Cleynen I, Engchuan W, Hestand MS, Heung T, Holleman AM, Johnston HR, Monfeuga T, McDonald-McGinn DM, Gur RE, Morrow BE, Swillen A, Vorstman JAS, Bearden CE, Chow EWC, van den Bree M, Emanuel BS, Vermeesch JR, Warren ST, Owen MJ, Chopra P, Cutler DJ, Duncan R, Kotlar A V, Mulle JG, Voss AJ, Zwick ME, Diacou A, Golden A, Guo T, Lin J-R, Wang T, Zhang Z, Zhao Y, Marshall C, Merico D, Jin A, Lilley B, Salmons HI, Tran O, Holmans P, Pardinas A, Walters JTR, Demaerel W, Boot E, Butcher NJ, Costain GA, Lowther C, Evers R, van Amelsvoort TAMJ, van Duin E, Vingerhoets C, Breckpot J, Devriendt K, Vergaelen E, Vogels A, Crowley TB, McGinn DE, Moss EM, Sharkus RJ, Unolt M, Zackai EH, Calkins ME, Gallagher RS, Gur RC, Tang SX, Fritsch R, Ornstein C, Repetto GM, Breetvelt E, Duijff SN, Fiksinski A, Moss H, Niarchou M, Murphy KC, Prasad SE, Daly EM, Gudbrandsen M, Murphy CM, Murphy DG, Buzzanca A, Fabio F Di, Digilio MC, Pontillo M, Marino B, Vicari S, Coleman K, Cubells JF, Ousley OY, Carmel M, Gothelf D, Mekori-Domachevsky E, Michaelovsky E, Weinberger R, Weizman A, Kushan L, Jalbrzikowski M, Armando M, Eliez S, Sandini C, Schneider M, Béna FS, Antshel KM, Fremont W, Kates WR, Belzeaux R, Busa T, Philip N, Campbell LE, McCabe KL, Hooper SR, Schoch K, Shashi V, Simon TJ, Tassone F, Arango C, Fraguas D, García-Miñaúr S, Morey-Canyelles J, Rosell J, Suñer DH, Raventos-Simic J, Epstein MP, Williams NM, Bassett AS, Consortium I 22q11. 2D. B and B (2020) Genetic contributors to risk of schizophrenia in the presence of a 22q11.2 deletion. Mol Psychiatry. https://doi.org/10.1038/s41380-020-0654-3

Collin G, Turk E, van den Heuvel MP (2016) Connectomics in Schizophrenia: From Early Pioneers to Recent Brain Network Findings. Biol psychiatry Cogn Neurosci neuroimaging 1:199–208 . https://doi.org/10.1016/j.bpsc.2016.01.002

Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131 . https://doi.org/https://doi.org/10.1016/j.brainres.2004.02.003

Consortium SWG of the PG (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Consortium TSWG of the PG, Ripke S, Walters JTR, O'Donovan MC (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. medRxiv 2020.09.12.20192922 . https://doi.org/10.1101/2020.09.12.20192922

Covelo A, Araque A (2018) Neuronal activity determines distinct gliotransmitter release from a single astrocyte. Elife 7: . https://doi.org/10.7554/eLife.32237

de Vrij FM, Bouwkamp CG, Gunhanlar N, Shpak G, Lendemeijer B, Baghdadi M, Gopalakrishna S, Ghazvini M, Li TM, Quadri M, Olgiati S, Breedveld GJ, Coesmans M, Mientjes E, de Wit T, Verheijen FW, Beverloo HB, Cohen D, Kok RM, Bakker PR, Nijburg A, Spijker AT, Haffmans PMJ, Hoencamp E, Bergink V, Vorstman JA, Wu T, Olde Loohuis LM, Amin N, Langen CD, Hofman A, Hoogendijk WJ, van Duijn CM, Ikram MA, Vernooij MW, Tiemeier H, Uitterlinden AG, Elgersma Y, Distel B, Gribnau J,

White T, Bonifati V, Kushner SA (2019) Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia. Mol Psychiatry 24:757–771 . https://doi.org/10.1038/s41380-017-0004-2

Dempster K, Norman R, Théberge J, Densmore M, Schaefer B, Williamson P (2015) Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. Psychiatry Res 233:180–185 . https://doi.org/10.1016/j.pscychresns.2015.06.003

Di Lorenzo G, Daverio A, Ferrentino F, Santarnecchi E, Ciabattini F, Monaco L, Lisi G, Barone Y, Di Lorenzo C, Niolu C, Seri S, Siracusano A (2015) Altered resting-state EEG source functional connectivity in schizophrenia: the effect of illness duration. Front Hum Neurosci 9:234 . https://doi.org/10.3389/fnhum.2015.00234

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. The lancet Psychiatry 7:272–281 . https://doi.org/10.1016/S2215-0366(19)30302-5

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proc Natl Acad Sci U S A 101:18206–18211 . https://doi.org/10.1073/pnas.0407976101

Doengi M, Hirnet D, Coulon P, Pape H-C, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca²⁺ signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci 106:17570 LP – 17575 . https://doi.org/10.1073/pnas.0809513106

Egerton A, Broberg B V, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, Perez-Iglesias R, Baandrup L, Düring SW, Sendt K V, Stone JM, Rostrup E, Sommer IE, Glenthøj B, Kahn RS, Dazzan P, McGuire P (2018) Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). Mol Psychiatry 23:2145–2155 . https://doi.org/10.1038/s41380-018-0082-9

Eide PK, Stanisic M (2010) Cerebral microdialysis and intracranial pressure monitoring in patients with idiopathic normal-pressure hydrocephalus: association with clinical response to extended lumbar drainage and shunt surgery. J Neurosurg 112:414–424 . https://doi.org/10.3171/2009.5.JNS09122

Falkenberg LE, Westerhausen R, Specht K, Hugdahl K (2012) Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. Proc Natl Acad Sci 109:5069 LP – 5073 . https://doi.org/10.1073/pnas.1115628109

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal Synchrony Mediated by Astrocytic Glutamate through Activation of Extrasynaptic NMDA Receptors. Neuron 43:729–743 . https://doi.org/https://doi.org/10.1016/j.neuron.2004.08.011

Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE (2002) Identification of the Mitochondrial Glutamate Transporter: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, AND TISSUE DISTRIBUTION OF TWO HUMAN ISOFORMS . J Biol Chem 277:19289–19294 . https://doi.org/10.1074/jbc.M201572200

Fleischhacker WW, Arango C, Arteel P, Barnes TRE, Carpenter W, Duckworth K, Galderisi S, Halpern L, Knapp M, Marder SR, Moller M, Sartorius N, Woodruff P (2014) Schizophrenia--time to commit to policy change. Schizophr Bull 40 Suppl 3:S165-94 . https://doi.org/10.1093/schbul/sbu006

Footz TK, Brinkman-Mills P, Banting GS, Maier SA, Riazi MA, Bridgland L, Hu S, Birren B, Minoshima S, Shimizu N, Pan H, Nguyen T, Fang F, Fu Y, Ray L, Wu H, Shaull S, Phan S, Yao Z, Chen F, Huan A, Hu P, Wang Q, Loh P, Qi S, Roe BA, McDermid HE (2001) Analysis of the cat eye syndrome critical region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere. Genome Res 11:1053–1070 . https://doi.org/10.1101/gr.154901

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. Biol Psychiatry 81:848–861 . https://doi.org/10.1016/j.biopsych.2017.03.005

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30:115–125 . https://doi.org/https://doi.org/10.1016/S0920-9964(97)00140-0

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, Ruderfer DM, Oh EC, Topol A, Shah HR, Klei LL, Kramer R, Pinto D, Gümüş ZH, Cicek AE, Dang KK, Browne A, Lu C, Xie L, Readhead B, Stahl EA, Xiao J, Parvizi M, Hamamsy T, Fullard JF, Wang Y-C, Mahajan MC, Derry JMJ, Dudley JT, Hemby SE, Logsdon BA, Talbot K, Raj T, Bennett DA, De Jager PL, Zhu J, Zhang B, Sullivan PF, Chess A, Purcell SM, Shinobu LA, Mangravite LM, Toyoshiba H, Gur RE, Hahn C-G, Lewis DA, Haroutunian V, Peters MA, Lipska BK, Buxbaum JD, Schadt EE, Hirai K, Roeder K, Brennand KJ, Katsanis N, Domenici E, Devlin B, Sklar P (2016) Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci 19:1442–1453 . https://doi.org/10.1038/nn.4399

Gallinat J, McMahon K, Kühn S, Schubert F, Schaefer M (2016) Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. Schizophr Bull 42:425–433 . https://doi.org/10.1093/schbul/sbv124

Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the Astroglial Glutamate Transporter, GLT-1, with Glycolytic Enzymes and Mitochondria. J Neurosci 31:18275 LP – 18288 . https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:e343 . https://doi.org/10.1371/journal.pbio.0040343

Glausier JR, Lewis DA (2013) Dendritic spine pathology in schizophrenia. Neuroscience 251:90–107 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2012.04.044

Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, Vadlamudi T, Abudulai F, Pham TT, Crocker A, Werner E, Wen Z, Repetto GM, Gogos JA, Claypool SM, Forsyth JK, Bearden CE, Glausier J, Lewis DA, Seyfried NT, Kwong JQ, Faundez V (2019) Systems Analysis of the 22q11.2 Microdeletion Syndrome Converges on a Mitochondrial Interactome Necessary for Synapse Function and Behavior. J Neurosci 39:3561 LP – 3581 . https://doi.org/10.1523/JNEUROSCI.1983-18.2019

Gonçalves VF, Giamberardino SN, Crowley JJ, Vawter MP, Saxena R, Bulik CM, Yilmaz Z, Hultman CM, Sklar P, Kennedy JL, Sullivan PF, Knight J (2018) Examining the role of common and rare mitochondrial variants in schizophrenia. PLoS One 13:e0191153–e0191153 . https://doi.org/10.1371/journal.pone.0191153

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344 . https://doi.org/10.1007/s11920-010-0124-8

Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957 . https://doi.org/10.1093/schbul/sbs010

Gordon A, Forsingdal A, Klewe IV, Nielsen J, Didriksen M, Werge T, Geschwind DH (2021) Transcriptomic networks implicate neuronal energetic abnormalities in three mouse models harboring autism and schizophrenia-associated mutations. Mol Psychiatry 26:1520–1534 . https://doi.org/10.1038/s41380-019-0576-0

Gottesman II (1991) Schizophrenia genesis: The origins of madness. W H Freeman/Times Books/ Henry Holt & Co, New York, NY, US

Goubert E, Mircheva Y, Lasorsa FM, Melon C, Profilo E, Sutera J, Becq H, Palmieri F, Palmieri L, Aniksztejn L, Molinari F (2017) Inhibition of the Mitochondrial Glutamate Carrier SLC25A22 in Astrocytes Leads to Intracellular Glutamate Accumulation. Front Cell Neurosci 11:149 . https://doi.org/10.3389/fncel.2017.00149

Goudriaan A, de Leeuw C, Ripke S, Hultman CM, Sklar P, Sullivan PF, Smit AB, Posthuma D, Verheijen MHG (2014) Specific Glial Functions Contribute to Schizophrenia Susceptibility. Schizophr Bull 40:925–935 . https://doi.org/10.1093/schbul/sbt109

Hans CP, Sharma N, Sen S, Zeng S, Dev R, Jiang Y, Mahajan A, Joshi T (2019) Transcriptomics Analysis Reveals New Insights into the Roles of Notch1 Signaling on Macrophage Polarization. Sci Rep 9:7999 . https://doi.org/10.1038/s41598-019-44266-4

Harrison G, Hopper K, Craig T, Laska E, Siegel C, Wanderling J, Dube KC, Ganev K, Giel R, an der Heiden W, Holmberg SK, Janca A, Lee PW, León CA, Malhotra S, Marsella AJ, Nakane Y, Sartorius N, Shen Y, Skoda C, Thara R, Tsirkin SJ, Varma VK, Walsh D, Wiersma D (2001) Recovery from psychotic illness: a 15- and 25-year international follow-up study. Br J Psychiatry 178:506–517 . https://doi.org/10.1192/bjp.178.6.506

Henriksen MG, Nordgaard J, Jansson LB (2017) Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. Front Hum Neurosci 11:322 . https://doi.org/10.3389/fnhum.2017.00322

Heston LL (1966) Psychiatric disorders in foster home reared children of schizophrenic mothers. Br J Psychiatry 112:819–825 . https://doi.org/10.1192/bjp.112.489.819

Hjelm BE, Rollins B, Mamdani F, Lauterborn JC, Kirov G, Lynch G, Gall CM, Sequeira A, Vawter MP (2015) Evidence of Mitochondrial Dysfunction within the Complex Genetic Etiology of Schizophrenia. Mol Neuropsychiatry 1:201–219 . https://doi.org/10.1159/000441252

Hoekstra SD, Stringer S, Heine VM, Posthuma D (2017) Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity . Front. Cell. Neurosci. 11:164

Hoffman RE, Dobscha SK (1989) Cortical Pruning and the Development of Schizophrenia: A Computer Model. Schizophr Bull 15:477–490 . https://doi.org/10.1093/schbul/15.3.477

Holmes DB, Heine VM (2017) Simplified 3D protocol capable of generating early cortical neuroepithelium. Biol Open 6:402–406 . https://doi.org/10.1242/bio.021725

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549–562 . https://doi.org/10.1093/schbul/sbp006

Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE (2004) Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J Neurosci 24:4551 LP – 4559 . https://doi.org/10.1523/JNEUROSCI.5217-03.2004

Hugdahl K, Craven AR, Nygård M, Løberg E-M, Berle JØ, Johnsen E, Kroken R, Specht K, Andreassen OA, Ersland L (2015) Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a (1)H MRS study. Schizophr Res 161:252–260 . https://doi.org/10.1016/j.schres.2014.11.015

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195–205 . https://doi.org/10.1016/0006-8993(79)90349-4

Iwamoto K, Bundo M, Kato T (2005) Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet 14:241–253 . https://doi.org/10.1093/hmg/ddi022

Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, Rogdaki M, Stone J, Egerton A, Turkheimer F, McGuire P, Howes OD (2018) The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. The Lancet Psychiatry 5:816–823 . https://doi.org/10.1016/S2215-0366(18)30268-2

Javitt DC, Hashim A, Sershen H (2005) Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 30:649–656 . https://doi.org/10.1038/sj.npp.1300589

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308 . https://doi.org/10.1176/ajp.148.10.1301

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22:1635–1648 . https://doi.org/10.1038/s41593-019-0486-0

Karlsgodt KH, van Erp TGM, Poldrack RA, Bearden CE, Nuechterlein KH, Cannon TD (2008) Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. Biol Psychiatry 63:512–518 . https://doi.org/10.1016/j.biopsych.2007.06.017

Karreman M, Moghaddam B (1996) The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. J Neurochem 66:589–598 . https://doi.org/10.1046/j.1471-4159.1996.66020589.x

Karry R, Klein E, Ben Shachar D (2004) Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 55:676–684 . https://doi.org/10.1016/j.biopsych.2003.12.012

Kathuria A, Lopez-Lengowski K, Jagtap SS, McPhie D, Perlis RH, Cohen BM, Karmacharya R (2020) Transcriptomic Landscape and Functional Characterization of Induced Pluripotent Stem Cell–Derived Cerebral Organoids in Schizophrenia. JAMA Psychiatry 77:745–754 . https://doi.org/10.1001/jamapsychiatry.2020.0196

Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, Andreassen OA, Arango C, Banaj N, Bouix S, Bousman CA, Brouwer RM, Bruggemann J, Bustillo J, Cahn W, Calhoun V, Cannon D, Carr V, Catts S, Chen J, Chen J-X, Chen X, Chiapponi C, Cho KK, Ciullo V, Corvin AS, Crespo-Facorro B, Cropley V, De Rossi P, Diaz-Caneja CM, Dickie EW, Ehrlich S, Fan F-M, Faskowitz J, Fatouros-Bergman H, Flyckt L, Ford JM, Fouche J-P, Fukunaga M, Gill M, Glahn DC, Gollub R, Goudzwaard ED, Guo H, Gur RE, Gur RC, Gurholt TP, Hashimoto R, Hatton SN, Henskens FA, Hibar DP, Hickie IB, Hong LE, Horacek J, Howells FM, Hulshoff Pol HE, Hyde CL, Isaev D, Jablensky A, Jansen PR, Janssen J, Jönsson EG, Jung LA, Kahn RS, Kikinis Z, Liu K, Klauser P, Knöchel C, Kubicki M, Lagopoulos J, Langen C, Lawrie S, Lenroot RK, Lim KO, Lopez-Jaramillo C, Lyall A, Magnotta V, Mandl RCW, Mathalon DH, McCarley RW, McCarthy-Jones S, McDonald C, McEwen S, McIntosh A, Melicher T, Mesholam-Gately RI, Michie PT, Mowry B, Mueller BA, Newell DT, O'Donnell P, Oertel-Knöchel V, Oestreich L, Paciga SA, Pantelis C, Pasternak O, Pearlson G, Pellicano GR, Pereira A, Pineda Zapata J, Piras F, Potkin SG, Preda A, Rasser PE, Roalf DR, Roiz R, Roos A, Rotenberg D, Satterthwaite TD, Savadjiev P, Schall U, Scott RJ, Seal ML, Seidman LJ, Shannon Weickert C, Whelan CD, Shenton ME, Kwon JS, Spalletta G, Spaniel F, Sprooten E, Stäblein M, Stein DJ, Sundram S, Tan Y, Tan S, Tang S, Temmingh HS, Westlye LT, Tønnesen S, Tordesillas-Gutierrez D, Doan NT, Vaidya J, van Haren NEM, Vargas CD, Vecchio D, Velakoulis D, Voineskos A, Voyvodic JQ, Wang Z, Wan P, Wei D, Weickert TW, Whalley H, White T, Whitford TJ, Wojcik JD, Xiang H, Xie Z, Yamamori H, Yang F, Yao N, Zhang G, Zhao J, van Erp TGM, Turner J, Thompson PM, Donohoe G (2018) Widespread white matter microstructural differences in schizophrenia across

4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry 23:1261–1269 . https://doi.org/10.1038/mp.2017.170

Kendler KS, Zerbin-Rüdin E (1996) Abstract and review of "Studien Uber Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin. Am J Med Genet 67:338–342 . https://doi.org/10.1002/(SICI)1096-8628(19960726)67:4<338::AID-AJMG4>3.0.CO;2-I

Kirov G, Rees E, Walters JTR, Escott-Price V, Georgieva L, Richards AL, Chambert KD, Davies G, Legge SE, Moran JL, McCarroll SA, O'Donovan MC, Owen MJ (2014) The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry 75:378–385 . https://doi.org/10.1016/j.biopsych.2013.07.022

Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder. JAMA Psychiatry 71:1323–1331 . https://doi.org/10.1001/jamapsychiatry.2014.1582

Kraepelin E (1990) Psychiatrie. 6 Auflage. Leipzig, Austria: Barth 1899. English translation by Metoui H, Ayed S: Psychiatry, A Textbook for Students and Physicians, Canton, MA

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, Charney DS (1994) Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. Arch Gen Psychiatry 51:199–214 . https://doi.org/10.1001/archpsyc.1994.03950030035004

Kumar P, Efstathopoulos P, Millischer V, Olsson E, Wei Y Bin, Brüstle O, Schalling M, Villaescusa JC, Ösby U, Lavebratt C (2018) Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. Sci Rep 8:12743 . https://doi.org/10.1038/s41598-018-31122-0

Lanillos P, Oliva D, Philippsen A, Yamashita Y, Nagai Y, Cheng G (2020) A review on neural network models of schizophrenia and autism spectrum disorder. Neural Netw 122:338–363 . https://doi.org/10.1016/j.neunet.2019.10.014

Leferink PS, Breeuwsma N, Bugiani M, van der Knaap MS, Heine VM (2018) Affected astrocytes in the spinal cord of the leukodystrophy vanishing white matter. Glia 66:862–873 . https://doi.org/10.1002/glia.23289

Lehmann D, Faber PL, Pascual-Marqui RD, Milz P, Herrmann WM, Koukkou M, Saito N, Winterer G, Kochi K (2014) Functionally aberrant electrophysiological cortical connectivities in first episode medication-naive schizophrenics from three psychiatry centers. Front Hum Neurosci 8:635 . https://doi.org/10.3389/fnhum.2014.00635

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci 113:E2685 LP-E2694 . https://doi.org/10.1073/pnas.1523717113

Lewis DA, Sweet RA (2009) Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J Clin Invest 119:706–716 . https://doi.org/10.1172/JCI37335

Li J, Ryan SK, Deboer E, Cook K, Fitzgerald S, Lachman HM, Wallace DC, Goldberg EM, Anderson SA (2019) Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia. Transl Psychiatry 9:302 . https://doi.org/10.1038/s41398-019-0643-y

Li Z, Hu M, Zong X, He Y, Wang D, Dai L, Dong M, Zhou J, Cao H, Lv L, Chen X, Tang J (2015) Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep 5:18553 . https://doi.org/10.1038/srep18553

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell 119:873–887 . https://doi.org/10.1016/j.cell.2004.11.003

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England) 373:234–239 . https://doi.org/10.1016/S0140-6736(09)60072-6

LUBY ED, GOTTLIEB JS, COHEN BD, ROSENBAUM G, DOMINO EF (1962) Model psychoses and schizophrenia. Am J Psychiatry 119:61–67 . https://doi.org/10.1176/ajp.119.1.61

Madhavan M, Nevin ZS, Shick HE, Garrison E, Clarkson-Paredes C, Karl M, Clayton BLL, Factor DC, Allan KC, Barbar L, Jain T, Douvaras P, Fossati V, Miller RH, Tesar PJ (2018) Induction of myelinating oligodendrocytes in human cortical spheroids. Nat Methods 15:700–706 . https://doi.org/10.1038/s41592-018-0081-4

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13:293–307 . https://doi.org/10.1038/nrn3229

Marder SR, Cannon TD (2019) Schizophrenia. N Engl J Med 381:1753–1761 . https://doi.org/10.1056/NEJMra1808803

Marwaha S, Johnson S, Bebbington P, Stafford M, Angermeyer MC, Brugha T, Azorin J-M, Kilian R, Hansen K, Toumi M (2007) Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br J Psychiatry 191:30–37 . https://doi.org/10.1192/bjp.bp.105.020982

Maurer I, Möller H-J (2004) Inhibition of complex I by neuroleptics in normal human brain cortex parallels the extrapyramidal toxicity of neuroleptics. Mol Cell Biochem 174:255–259

Maurer I, Zierz S, Möller H (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 48:125–136 . https://doi.org/10.1016/s0920-9964(00)00075-x

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-An Overview. JAMA psychiatry 77:201–210 . https://doi.org/10.1001/jamapsychiatry.2019.3360

Mederos S, González-Arias C, Perea G (2018) Astrocyte–Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function. Front Synaptic Neurosci 10: . https://doi.org/10.3389/fnsyn.2018.00045

Millar JK, James R, Christie S, Porteous DJ (2005) Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci 30:477–484 . https://doi.org/10.1016/j.mcn.2005.08.021

Miller G (2010) Psychiatry. Beyond DSM: seeking a brain-based classification of mental illness. Science 327:1437

Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA (2012) Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 17:1206–1227 . https://doi.org/10.1038/mp.2012.47

Modica-Napolitano JS, Lagace CJ, Brennan WA, Aprille JR (2003) Differential effects of typical and atypical neuroleptics on mitochondrial functionin vitro. Arch Pharm Res 26:951–959 . https://doi.org/10.1007/BF02980205

Molofsky A V, Krencik R, Ullian EM, Tsai H, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907 . https://doi.org/10.1101/gad.188326.112

Montes de Oca Balderas P (2018) Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. Int J Mol Sci 19:3800 . https://doi.org/10.3390/ijms19123800

Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC (2017) Derivation of Human Midbrain-Specific Organoids from Neuroepithelial Stem Cells. Stem cell reports 8:1144–1154 . https://doi.org/10.1016/j.stemcr.2017.03.010

Morató L, Bertini E, Verrigni D, Ardissone A, Ruiz M, Ferrer I, Uziel G, Pujol A (2014) Mitochondrial dysfunction in central nervous system white matter disorders. Glia 62:1878–1894 . https://doi.org/10.1002/glia.22670

Mouaffak F, Kebir O, Bellon A, Gourevitch R, Tordjman S, Viala A, Millet B, Jaafari N, Olié JP, Krebs MO (2011) Association of an UCP4 (SLC25A27) haplotype with ultra-resistant schizophrenia. Pharmacogenomics 12:185–193 . https://doi.org/10.2217/pgs.10.179

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SHR (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. Nat Neurosci 18:219–226 . https://doi.org/10.1038/nn.3901

Nadadhur AG, Leferink PS, Holmes D, Hinz L, Cornelissen-Steijger P, Gasparotto L, Heine VM (2018) Patterning factors during neural progenitor induction determine regional identity and differentiation potential in vitro. Stem Cell Res 32:25–34 . https://doi.org/https://doi.org/10.1016/j.scr.2018.08.017

Nakao K, Jeevakumar V, Jiang SZ, Fujita Y, Diaz NB, Pretell Annan CA, Eskow Jaunarajs KL, Hashimoto K, Belforte JE, Nakazawa K (2019) Schizophrenia-Like Dopamine Release Abnormalities in a Mouse Model of NMDA Receptor Hypofunction. Schizophr Bull 45:138–147 . https://doi.org/10.1093/schbul/sby003

Ni P, Chung S (2020) Mitochondrial Dysfunction in Schizophrenia. Bioessays 42:e1900202 . https://doi.org/10.1002/bies.201900202

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23–45 . https://doi.org/10.1007/978-1-61779-452-0_3

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of Adult Human Astrocytes. J Neurosci 29:3276 LP – 3287 . https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553 . https://doi.org/10.1016/j.tins.2006.08.004

Ohtani T, Bouix S, Hosokawa T, Saito Y, Eckbo R, Ballinger T, Rausch A, Melonakos E, Kubicki M (2014) Abnormalities in white matter connections between orbitofrontal cortex and anterior cingulate cortex and their associations with negative symptoms in schizophrenia: A DTI study. Schizophr Res 157:190–197 . https://doi.org/https://doi.org/10.1016/j.schres.2014.05.016

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H (2012) Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation. Acta Psychiatr Scand 126:72–78 . https://doi.org/10.1111/j.1600-0447.2012.01853.x

Ozcelik-Eroglu E, Ertugrul A, Oguz KK, Has AC, Karahan S, Yazici MK (2014) Effect of clozapine on white matter integrity in patients with schizophrenia: a diffusion tensor imaging study. Psychiatry Res 223:226–235 . https://doi.org/10.1016/j.pscychresns.2014.06.001

Park Y-U, Jeong J, Lee H, Mun JY, Kim J-H, Lee JS, Nguyen MD, Han SS, Suh P-G, Park SK (2010) Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107:17785–17790 . https://doi.org/10.1073/pnas.1004361107

Paşca SP, Portmann T, Voineagu I, Yazawa M, Shcheglovitov A, Paşca AM, Cord B, Palmer TD, Chikahisa S, Nishino S, Bernstein JA, Hallmayer J, Geschwind DH, Dolmetsch RE (2011) Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 17:1657–1662 . https://doi.org/10.1038/nm.2576

Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A (2016) Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife 5: . https://doi.org/10.7554/eLife.20362

Pirttimaki TM, Hall SD, Parri HR (2011) Sustained Neuronal Activity Generated by Glial Plasticity. J Neurosci 31:7637 LP – 7647 . https://doi.org/10.1523/JNEUROSCI.5783-10.2011

Pita-Almenar JD, Collado MS, Colbert CM, Eskin A (2006) Different Mechanisms Exist for the Plasticity of Glutamate Reuptake during Early Long-Term Potentiation (LTP) and Late LTP. J Neurosci 26:10461 LP – 10471 . https://doi.org/10.1523/JNEUROSCI.2579-06.2006

Pita-Almenar JD, Zou S, Colbert CM, Eskin A (2012) Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. Learn Mem 19:615–626 . https://doi.org/10.1101/lm.023259.111

Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19:20–29 . https://doi.org/10.1038/mp.2013.136

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675 LP-E2684 . https://doi.org/10.1073/pnas.1520759113

Regenold WT, Pratt M, Nekkalapu S, Shapiro PS, Kristian T, Fiskum G (2012) Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: implications for brain energy metabolism and neurotrophic signaling. J Psychiatr Res 46:95–104 . https://doi.org/10.1016/j.jpsychires.2011.09.018

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PKE, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman P V, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang K-Y, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CNA, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CCA, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CCA, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF, Consortium MGS of S, Consortium PEI, 2 WTCCC, Committee: M, Group: D and A, DNA Data QC and Informatics Group: G, Committee: P (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150–1159 . https://doi.org/10.1038/ng.2742

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517 . https://doi.org/10.1126/science.273.5281.1516

Robicsek O, Ene HM, Karry R, Ytzhaki O, Asor E, McPhie D, Cohen BM, Ben-Yehuda R, Weiner I, Ben-Shachar D (2018) Isolated Mitochondria Transfer Improves Neuronal Differentiation of Schizophrenia-Derived Induced Pluripotent Stem Cells and Rescues Deficits in a Rat Model of the Disorder. Schizophr Bull 44:432–442 . https://doi.org/10.1093/schbul/sbx077

Robicsek O, Karry R, Petit I, Salman-Kesner N, Müller F-J, Klein E, Aberdam D, Ben-Shachar D (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry 18:1067–76 . https://doi.org/10.1038/mp.2013.67

Robinson DG, Woerner MG, McMeniman M, Mendelowitz A, Bilder RM (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. Am J Psychiatry 161:473–479 . https://doi.org/10.1176/appi.ajp.161.3.473

Romanos, J., Benke, D., Saab, A.S. et al. Differences in glutamate uptake between cortical regions impact neuronal NMDA receptor activation. Commun Biol 2, 127 (2019). https://doi.org/10.1038/s42003-019-0367-9

Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M (2014) White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. J Neuroimaging 24:101–110 . https://doi.org/10.1111/j.1552-6569.2012.00779.x

Satrústegui J, Pardo B, Del Arco A (2007) Mitochondrial transporters as novel targets for intracellular calcium signaling. Physiol Rev 87:29–67 . https://doi.org/10.1152/physrev.00005.2006

Sawada T, Chater TE, Sasagawa Y, Yoshimura M, Fujimori-Tonou N, Tanaka K, Benjamin KJM, Paquola ACM, Erwin JA, Goda Y, Nikaido I, Kato T (2020) Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. Mol Psychiatry 25:2695–2711 . https://doi.org/10.1038/s41380-020-0844-z

Schrode N, Ho S-M, Yamamuro K, Dobbyn A, Huckins L, Matos MR, Cheng E, Deans PJM, Flaherty E, Barretto N, Topol A, Alganem K, Abadali S, Gregory J, Hoelzli E, Phatnani H, Singh V, Girish D, Aronow B, Mccullumsmith R, Hoffman GE, Stahl EA, Morishita H, Sklar P, Brennand KJ (2019) Synergistic effects of common schizophrenia risk variants. Nat Genet 51:1475–1485 . https://doi.org/10.1038/s41588-019-0497-5

Schulmann A, Ryu E, Goncalves V, Rollins B, Christiansen M, Frye MA, Biernacka J, Vawter MP (2019) Novel Complex Interactions between Mitochondrial and Nuclear DNA in Schizophrenia and Bipolar Disorder. Mol neuropsychiatry 5:13–27 . https://doi.org/10.1159/000495658

Shaker MR, Pietrogrande G, Martin S, Lee J-H, Sun W, Wolvetang EJ (2021) Rapid and Efficient Generation of Myelinating Human Oligodendrocytes in Organoids. Front Cell Neurosci 15:631548 . https://doi.org/10.3389/fncel.2021.631548

Shao L, Martin M V, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP (2008) Mitochondrial involvement in psychiatric disorders. Ann Med 40:281–295 . https://doi.org/10.1080/07853890801923753

Shao Z, Noh H, Bin Kim W, Ni P, Nguyen C, Cote SE, Noyes E, Zhao J, Parsons T, Park JM, Zheng K, Park JJ, Coyle JT, Weinberger DR, Straub RE, Berman KF, Apud J, Ongur D, Cohen BM, McPhie DL, Rapoport JL, Perlis RH, Lanz TA, Xi HS, Yin C, Huang W, Hirayama T, Fukuda E, Yagi T, Ghosh S, Eggan KC, Kim H-Y, Eisenberg LM, Moghadam AA, Stanton PK, Cho J-H, Chung S (2019) Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci 22:229–242 . https://doi.org/10.1038/s41593-018-0313-z

Shergill SS, Kanaan RA, Chitnis XA, O'Daly O, Jones DK, Frangou S, Williams SCR, Howard RJ, Barker GJ, Murray RM, McGuire P (2007) A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 164:467–473 . https://doi.org/10.1176/ajp.2007.164.3.467

Sheng, Z. H., & Cai, Q. (2012). Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. Nature reviews. Neuroscience, 13(2), 77–93. https://doi.org/10.1038/nrn3156

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618 LP – 5623 . https://doi.org/10.1073/pnas.0501559102

Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang W, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai L-H (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18:1008–1016 . https://doi.org/10.1038/nn.4023

Sigurdsson T (2016) Neural circuit dysfunction in schizophrenia: Insights from animal models. Neuroscience 321:42–65 . https://doi.org/https://doi.org/10.1016/j.neuroscience.2015.06.059

Somerville SM, Lahti AC, Conley RR, Roberts RC (2011) Mitochondria in the striatum of subjects with schizophrenia: Relationship to treatment response. Synapse 65:215–224 . https://doi.org/10.1002/syn.20838

Stilo SA, Murray RM (2019) Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep 21:100 . https://doi.org/10.1007/s11920-019-1091-3

Stone JM, Howes OD, Egerton A, Kambeitz J, Allen P, Lythgoe DJ, O'Gorman RL, McLean MA, Barker GJ, McGuire P (2010) Altered Relationship Between Hippocampal Glutamate Levels and Striatal Dopamine Function in Subjects at Ultra High Risk of Psychosis. Biol Psychiatry 68:599–602 . https://doi.org/10.1016/j.biopsych.2010.05.034

Sullivan EM, O'Donnell P (2012) Inhibitory Interneurons, Oxidative Stress, and Schizophrenia. Schizophr Bull 38:373–376 . https://doi.org/10.1093/schbul/sbs052

Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet 13:537–551 . https://doi.org/10.1038/nrg3240

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192 . https://doi.org/10.1001/archpsyc.60.12.1187

Sun H, Lui S, Yao L, Deng W, Xiao Y, Zhang W, Huang X, Hu J, Bi F, Li T, Sweeney JA, Gong Q (2015) Two Patterns of White Matter Abnormalities in Medication-Naive Patients With First-Episode Schizophrenia Revealed by Diffusion Tensor Imaging and Cluster Analysis. JAMA Psychiatry 72:678–686 . https://doi.org/10.1001/jamapsychiatry.2015.0505

Szabo A, Akkouh IA, Vandenberghe M, Osete JR, Hughes T, Heine V, Smeland OB, Glover JC, Andreassen OA, Djurovic S (2021) A human iPSC-astroglia neurodevelopmental model reveals divergent transcriptomic patterns in schizophrenia. Transl Psychiatry 11:554 . https://doi.org/10.1038/s41398-021-01681-4

Tansey KE, Rees E, Linden DE, Ripke S, Chambert KD, Moran JL, McCarroll SA, Holmans P, Kirov G, Walters J, Owen MJ, O'Donovan MC (2016) Common alleles contribute to schizophrenia in CNV carriers. Mol Psychiatry 21:1085–1089 . https://doi.org/10.1038/mp.2015.143

Tarasov V V, Svistunov AA, Chubarev VN, Sologova SS, Mukhortova P, Levushkin D, Somasundaram SG, Kirkland CE, Bachurin SO, Aliev G (2020) Alterations of Astrocytes in the Context of Schizophrenic Dementia. Front Pharmacol 10: . https://doi.org/10.3389/fphar.2019.01612

Tcw J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem cell reports 9:600–614 . https://doi.org/10.1016/j.stemcr.2017.06.018

Topol A, English JA, Flaherty E, Rajarajan P, Hartley BJ, Gupta S, Desland F, Zhu S, Goff T, Friedman L, Rapoport J, Felsenfeld D, Cagney G, Mackay-Sim A, Savas JN, Aronow B, Fang G, Zhang B, Cotter D, Brennand KJ (2015) Increased abundance of translation machinery in stem cell-derived neural progenitor cells from four schizophrenia patients. Transl Psychiatry 5:e662–e662 . https://doi.org/10.1038/tp.2015.118

Toyoshima M, Akamatsu W, Okada Y, Ohnishi T, Balan S, Hisano Y, Iwayama Y, Toyota T, Matsumoto T, Itasaka N, Sugiyama S, Tanaka M, Yano M, Dean B, Okano H, Yoshikawa T (2016) Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl Psychiatry 6:e934 . https://doi.org/10.1038/tp.2016.206

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497–502 . https://doi.org/10.1097/JCP.0b013e3182214aad

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610 . https://doi.org/https://doi.org/10.1016/S0361-9230(01)00528-7

Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, Hamilton EG, Mitjans M, Maddalena G, Begemann M, Seidel J, Yanamori H, Jaffe AE, Berman KF, Egan MF, Straub RE, Colantuoni C, Blasi G, Hashimoto R, Rujescu D, Ehrenreich H, Bertolino A, Weinberger DR (2018) Convergence of placenta biology and genetic risk for schizophrenia. Nat Med 24:792–801 . https://doi.org/10.1038/s41591-018-0021-y

Vasile F, Dossi E, Rouach N (2017) Human astrocytes: structure and functions in the healthy brain. Brain Struct Funct 222:2017–2029 . https://doi.org/10.1007/s00429-017-1383-5

Verge B, Alonso Y, Miralles C, Valero J, Vilella E, Boles RG, Martorell L (2012) New evidence for the involvement of mitochondrial inheritance in schizophrenia: results from a cross-sectional study evaluating the risk of illness in relatives of schizophrenia patients. J Clin Psychiatry 73:684—690 . https://doi.org/10.4088/jcp.10m06718

Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017) Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826 . https://doi.org/10.1038/s41467-017-01261-5

Wei Y, de Lange SC, Scholtens LH, Watanabe K, Ardesch DJ, Jansen PR, Savage JE, Li L, Preuss TM, Rilling JK, Posthuma D, van den Heuvel MP (2019) Genetic mapping and evolutionary analysis of human-expanded cognitive networks. Nat Commun 10:4839 . https://doi.org/10.1038/s41467-019-12764-8

Welle S, Bhatt K, Shah B, Needler N, Delehanty JM, Thornton CA (2003) Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94:1479–1484 . https://doi.org/10.1152/japplphysiol.01061.2002

Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y, Kim N-S, Yoon K-J, Shin J, Zhang C, Makri G, Nauen D, Yu H, Guzman E, Chiang C-H, Yoritomo N, Kaibuchi K, Zou J, Christian KM, Cheng L, Ross CA, Margolis RL, Chen G, Kosik KS, Song H, Ming G (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515:414–418 . https://doi.org/10.1038/nature13716

Wender PH, Rosenthal D, Kety SS, Schulsinger F, Welner J (1974) Crossfostering. A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. Arch Gen Psychiatry 30:121–128 . https://doi.org/10.1001/archpsyc.1974.01760070097016

Wessman J, Paunio T, Tuulio-Henriksson A, Koivisto M, Partonen T, Suvisaari J, Turunen JA, Wedenoja J, Hennah W, Pietiläinen OPH, Lönnqvist J, Mannila H, Peltonen L (2009) Mixture model clustering

of phenotype features reveals evidence for association of DTNBP1 to a specific subtype of schizophrenia. Biol Psychiatry 66:990–996 . https://doi.org/10.1016/j.biopsych.2009.05.034

Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017) Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem Cell 21:195-208.e6 . https://doi.org/10.1016/j.stem.2017.06.012

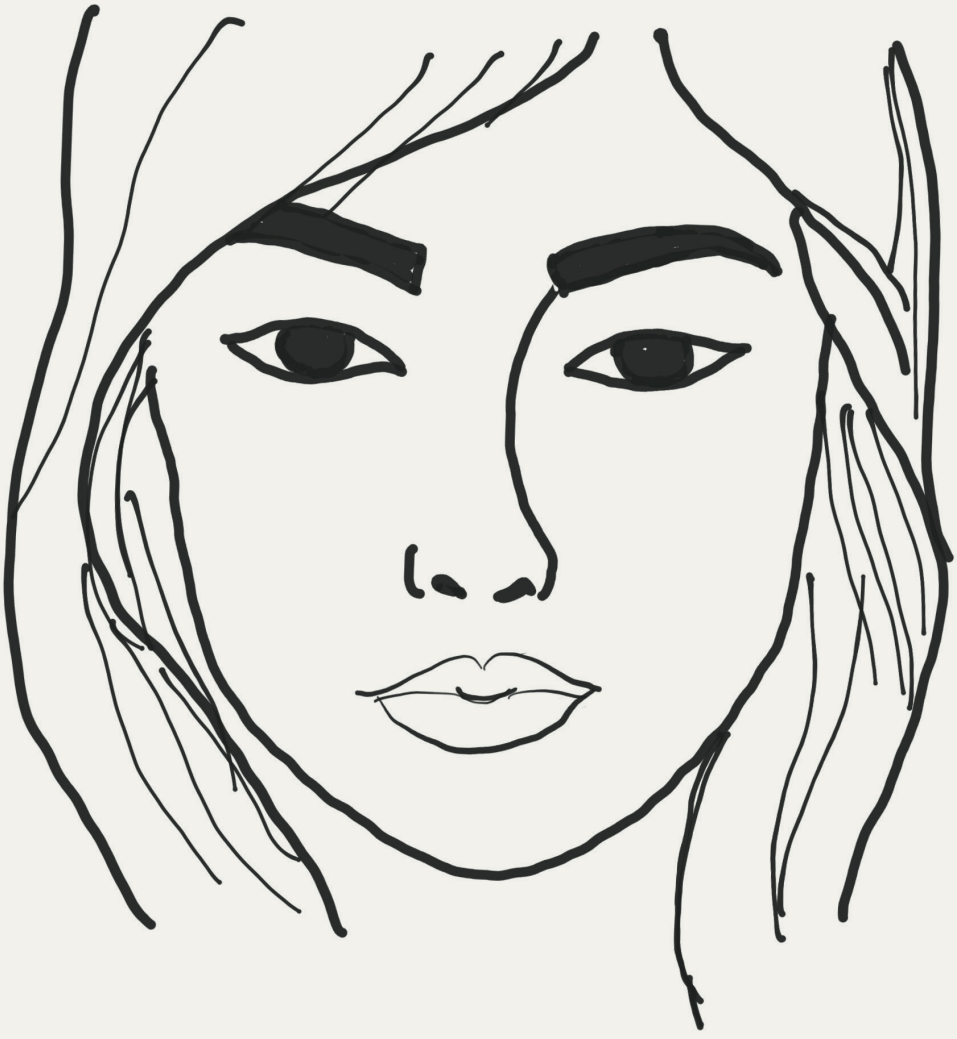
Wong L-JC (2012) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61 . https://doi.org/10.1055/s-0032-1306387

Xiao Y, Sun H, Shi S, Jiang D, Tao B, Zhao Y, Zhang W, Gong Q, Sweeney JA, Lui S (2018) White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia. Am J Psychiatry 175:1129–1136 . https://doi.org/10.1176/appi.ajp.2018.17121402

Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, Ongur D, McPhie D, Cohen B, Perlis R, Tsai L-H (2015) Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. Mol Psychiatry 20:162–169 . https://doi.org/10.1038/mp.2014.143

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34:11929–11947 . https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193 LP – 1198 . https://doi.org/10.1073/pnas.1119675109



Appendix

Dutch Summary
Publications
Acknowledgements

NEDERLANDSE SAMENVATTING

Schizofrenie is een ernstige ontwikkelingsstoornis die meestal tot uiting komt in adolescentie. Het wordt gekenmerkt door meerder symptomen die onderverdeeld worden in 3 categorieën: positieve symptomen (bijvoorbeeld hallucinaties), cognitieve symptomen (zoals verminderd geheugen) en negatieve symptomen (zoals sociale terugtrekking). We weten dat de aard van schizofrenie voor een groot deel genetisch is, maar er spelen ook omgevingsfactoren mee. De genetische architectuur van deze ziekte is uiterst complex, en bestaat uit naar schatting duizenden genen. Daarom is et lastig om schizofrenie in diermodellen na te bootsen. Met het gebruik van geïnduceerd pluripotente stamcellen, kunnen we wel deze complexe genetische samenstelling behouden, omdat deze cellen genetisch identiek zijn aan de donor. In het huidige proefschrift, hebben we huidcellen van schizofreniepatiënten en van gezonde proefpersonen gebruikt die we hebben gereprogrammeerd tot geïnduceerd pluripotente stamcellen. Deze stamcellen hebben we vervolgens gedifferentieerd tot hersencellen om afwijkingen te detecteren tussen gezonde hersencellen en hersencellen van schizofreniepatiënten.

In Hoofdstuk 1 is ten eerste de geschiedenis van de ziekte samengevat. Verder wordt hier de klinische aspecten en genetische complexiteit besproken. Er zijn verschillende theorieën over de biologische oorzaken van schizofrenie, die ook in dit hoofdstuk worden uiteengezet. Uiteraard bevat dit hoofdstuk ook een overzicht van de diermodellen en de bevindingen die door anderen zijn gedaan voorgaand aan dit proefschrift.

In Hoofdstuk 2 wordt besproken hoe je patiënten en gezonde proefpersonen kan selecteren op basis van hun genetisch profiel. Door gebruik te maken van polygene risico scores, is men in staat om de twee extremen te selecteren om op deze wijze de hoogst haalbare statistische power te verkrijgen om potentiele verschillen tussen deze groepen te detecteren.

In Hoofdstuk 3 bevat data over twee verschillende reprogrammeringsmethoden: episomale reprogrammering en Lentivirale reprogrammering. Hier wordt onderzocht welke optie het meest geschikt is om cellen te reprogrammeren om onderzoek te doen naar genetisch complexe ziekten. Het feit dat episomale reprogramming niet tot integratie van DNA-materiaal in het genoom van de gastheer leidt is een groot voordeel aangezien men niet ziekte geassocieerde genen wil beïnvloeden. Door gebruik te maken van cellen van Rett- patiënten zien we echter wel verschillen tussen deze methoden als vrouwelijke cellen worden gebruikt: lentiviralle reprogrammering leidt tot reactivatie van het tweede X-chromosoom die in volwassen stadium is uitgeschakeld. Dit zien we niet wanneer dezelfde cellen worden gereprogrammeerd door episomale vectoren. Er zijn echter geen verdere verschillen in pluripotentie scores op basis van de PluriTest. Episomale reprogrammering

kan worden gebruikt bij het produceren van isogene vrouwelijke lijnen van patiënten met een X-chromosomale ziekte. Verder laat dit hoofdstuk zien, dat we voor de schizofrenielijnen episomale reprogrammering kunnen gebruiken.

In Hoofdstuk 4 tonen we de variatie tussen cellijnen. Door van 3 proefpersonen stamcellen te genereren en die weer te differentiëren (allen in parallel) tonen we aan de hand van eiwitexpressie dat de meeste variatie wordt geïntroduceerd door genetische achtergrond van de verschillende donoren. Verschillende lijnen van dezelfde donor lijken meer op elkaar dan op lijnen van een andere donor. Het gebuikte differentiatie protocol leidt tot de minste variatie. Aan de hand van deze data, tonen we aan de hand van een simulatie aan dat het gebruik van meerdere donoren een veel groter voordeel oplevert voor de statistische power van een studie dan het gebruik van meerdere cellijnen van dezelfde donor. Dit geeft ons nuttige informatie voor de opzet van ons schizofrenieonderzoek.

In Hoofdstuk 5 gebruiken we de informatie van de voorgaande hoofdstukken en vergelijken we cellen van schizofreniepatiënten en van gezonde proefpersonen. We zien hier dat in astrocyten, 10 genen anders tot expressie komen in schizofreniepatiënten. Van deze genen onderzoeken we SLC25A18. Dit is een mitochondriaal eiwit die niet eerder in verband is gebracht met schizofrenie. We tonen ook aan dat astrocyten van patiënten invloed hebben op de netwerkformatie van gezonde neuronen, waarbij de laatstgenoemden minder inhibitoire synapsen maken. Dit leidt tot een imbalans tussen exitatoire en inhibitoire synapsen, die we ook in patiënten en diermodellen zien. Wellicht is hier een rol voor mitochondriale defecten.

In Hoofdstuk 6 onderzoeken we het gebruik van een complexer model voor schizofrenie onderzoek, waarbij we in 3D kweken. We zien hier functionele neuronen, astrocyten en myelinerende oligodendrocyten. Dit vormt een completer en meer fysiologisch model voor het brein. We brengen de tijdlijn in maturatie van de cellen in kaart en onderzoeken verschillen tussen organoïden van patiënten en gezonde proefpersonen. Helaas leidt de vergrote complexiteit van dit model ook tot meer variatie, waardoor verschillen moeilijker zijn op te pikken. Daarnaast is verdere optimalisatie van analytische methoden noodzakelijk.

In Hoofdstuk 7 worden de resultaten van het huidige proefschrift vergeleken met andere bestaande data over schizofrenie. Hier wordt besproken dat mitochondriale afwijkingen vaak zijn gerapporteerd in schizofrenie en wellicht ook gelinkt kunnen zijn met de verschillende theorieën over de biologische oorzaken van schizofrenie. Verder worden hier nog suggesties gedaan voor vervolgonderzoek.

PUBLICATIONS

Hoekstra SD, Stringer S, Heine VM, Posthuma D. Genetically-Informed Patient Selection for iPSC Studies of Complex Diseases May Aid in Reducing Cellular Heterogeneity. Front Cell Neurosci . 2017;11:164. http://journal.frontiersin.org/article/10.3389/fncel.2017.00164.

Hinz L, **Hoekstra SD**, Watanabe K, Posthuma D, Heine VM. Generation of Isogenic Controls for In Vitro Disease Modelling of X-Chromosomal Disorders. Stem Cell Rev Reports. 2018. doi:10.1007/s12015-018-9851-8

Wong MMK, **Hoekstra SD**, Vowles J, et al. Neurodegeneration in SCA14 is associated with increased PKCγ kinase activity, mislocalization and aggregation. Acta Neuropathol Commun. 2018;6(1):99. doi:10.1186/s40478-018-0600-7

Beekhuis-Hoekstra SD, Watanabe K, Werme J, et al. Systematic assessment of variability in the proteome of iPSC derivatives. Stem Cell Res. 2021;56:102512. doi:10.1016/j.scr.2021.102512

DANKWOORD

Eindelijk is het zo ver! Mijn proefschrift is af. Er zijn veel mensen die direct en indirect hebben bijgedragen aan dit boekje. Als eerste wil graag mijn promotoren, Danielle en Vivi bedanken voor deze unieke mogelijkheid om te promoveren op iPSCs in schizofrenie (dat was precies wat ik graag wilde). Binnen het CTG en de stamcel lab heb ik ontzettend veel geleerd over celkweek, stamcellen, genetica en statistiek maar heb ik ook veel vrienden gemaakt. Danielle, je hebt een een ontzettende goeie sfeer gecreëerd binnen de groep, waardoor iedereen benaderbaar is en veel collega's voelen als vrienden. Vivi, je bent zowel professioneel als persoonlijk erg betrokken bij iedereen in de groep wat ik heel erg bijzonder vind en ontzettend waardeer. Je denkt graag mee, en helpt altijd bij het vinden van de juiste persoon: 'Oh, dan lopen we gelijk even langs!'. En jouw persoonlijke betrokkenheid was erg zichtbaar tijdens mijn zwangerschap en toen ik advies kwam vragen over mijn verdere carrière. Dankzij jou ben ik bij het MCBI beland! Dank je wel.

Of course I would also like to thank my committee members prof.dr. Martijn van den Heuvel, prof.dr. Elga de Vries, prof.dr. Elly Hol, prof.dr. Srdjan Djurovic en dr. Rogier Min for taking the time to read an evaluate this dissertation.

In het stamcel lab waren er veel mensen die me veel hebben geholpen. Gerbren werkte altijd geduldig nieuwe mensen in, met veel structuur, geduld en humor. Het structurele is ook duidelijk te zien in jouw mapjes in mapjes in mapjes, etc. Ik moet jou uiteraard ook bedanken voor de Netflix account! Aish, thank you for always answering my questions about your neuronal protocol, for the joy you have brought to the lab and for the sari! Prisca, erg bedankt voor je hulp bij het Israël protocol en het openen van de dansvloer. Dwayne, thank you for all the talks about a very wide variety of topics during lunch! I must say that most of the conversations we've had were very insightful. I must thank Lisa G for always being there to help whenever I had troubles (both professional and personal) and giving great advice. You are by far the most patient person I have ever met! Like an Italian angel with amazing hair who makes excellent tiramisu. Paulien, heel erg bedankt voor je hulp met de western blots en alle babypraat. Ik hoop dat alles goed gaat met jou en jouw kleinkinderen! Anna, dank je wel voor de les in hoepelen en voor de gezellige pole flow les op een blauwe maandag. En dan wordt mijn project overgenomen door Karen! Ik vond het erg moeilijk om 'mijn lijntjes' over te dragen aangezien ik er zo lang mee bezig was. Gelukkig zijn ze nu in goede handen, en ik ben heel blij dat het onderzoek verder gaat! Ook anderen in het stamcel lab hebben uiteraard bijgedragen door hun input tijdens meetings en gezelligheid. Anne, bedankt voor jouw humor en sarcasme. Mijn naamgenote Stephanie D, de vaste rots van het stamcel lab. Claudia, whom I'm grateful for all the talks about (Italian) food! Ook dank aan Kevin, Liza, Lauria en Roberto.

Bij mijn eerste dagen in het CTG was Anke degene die me in het begin op sleeptouw mee nam. Dat deed je geloof ik bij alle nieuwkomers! Met jouw warme persoonlijkheid zorgde jij ervoor dat iedereen zich welkom voelde, en je was er altijd als men zijn problemen en frustraties wilde bespreken. Ook Mats heeft veel van mijn frustraties moeten aanhoren, zeker als kantoorgenoot aan het einde van onze PhD. Gelukkig ben je erg betrokken bij je collega's en ben jij de definitie van 'chille gast': er is geloof ik werkelijk niemand die niet met jou door 1 deur kan. Dan hebben we de statistiek goeroe Christiaan. Je maakt een behoorlijke indruk met jouw kennis en iedereen klopt bij jou aan voor advies. Gelukkig had je ook tijd voor mijn domme vragen en kon je ingewikkelde statistische concepten goed uitleggen. Het liefst natuurlijk onder genot van een single malt whiskey, als trouwe vrijdagmiddag borrelaar. The other guru in the lab was Kyoko. You effectively combine crazy hard work with incredible intelligence and luckily you were always very helpful and polite whenever I asked something basic. Thank you so much for all you help with the data and for all the fun we had together, usually after some wine! Another brilliant mind is Josephine, whom I'm particularly grateful for the simulation of the 'pilot' and for your joy during the Friday drinks and lab outings. Ik wil ook graag Eva bedanken voor jouw gezelligheid tijdens borrels en de lunch! Je bent altijd erg geïnteresseerd en sociaal. Het was ook erg leuk dat de groep Martijn erbij kwam! Dirk-Jan (the DJ), Elleke, Rory, Siemon, Doug en Lianne met jullie werden de lunch en borrels nog gezelliger. Yongbin, thank you for the expression data! Sofie, bedankt voor al je hulp met statistiek. Je bent altijd geduldig en bijzonder behulpzaam. Met Iris kon ik altijd erg gezellig praten over baby's en ook jij kon goed borrelen! Eline, je was altijd vrolijk! erg bedankt voor al je ondersteunende hulp en voor alle gezellige praatjes zo maar spontaan midden op de dag. Ook met jou kon ik lekker over de kinderen praten. Ik hoop dat alles goed gaat met jou en je gezinnetje.

Ook mijn huidige groepsleiders Elga en Sue wil ik graag bedanken voor al hun advies en voor alle mogelijkheden die ik nu krijg. SkinLab en Neuroimmunology groups, thank you for the warm welcome and your interest in stem cells!

Of course I would like to take this opportunity to extensively thank my paranimphs & dearest friends Lisa H and Celine. Lisa, you are my lab sister, since we started in the lab together. I was impressed with your personality and your knowledge. We shared many of the same problems and frustrations and you were always there to listen and to help. We shared much more than our PhD lives: we started to go to the gym together, having dinners, borrels and we even had our weddings one week apart. Thank you for always being there. Toen kwam genie Céline bij de groep! Céline, je bent een topwijf… je bent ontzettend intelligent, soms pijnlijk eerlijk maar vooral erg lief. Ik vind het erg knap hoe jij door je PhD walst alsof het niks is en daarnaast nog een baby grootbrengt en ook al je hobby's andere werkzaamheden weet te

volbrengen. Chapeau! My PhD would have been so boring without both of you. We started off as colleagues and became close friends and I am very greatful for that.

Uiteraard wil ik mijn familie bedanken! Als eerst natuurlijk mijn ouders, die deze kans hebben gegeven door mij een superfijne jeugd te geven en alle mogelijkheden die ik heb gehad. Ik kan altijd op jullie rekenen. Ook Maarten en Nathalie, zonder jullie was die fijne jeugd een stuk saaier geweest. En jullie begrepen goed hoe frustrerend de vraag “Wanneer ben je nou klaar met je ‘studie’?” was.
Opa, bedankt voor al je lovende woorden en je hulp bij het printen van dit proefschrift! Jij bent altijd degene die overduidelijk de meeste moeite doet om precies te begrijpen wat ik nou allemaal aan het uitvreten ben in het lab. Chris, dank je wel voor al je hulp met het oppassen op de meiden! Zonder jou, Ma en Anita zou dit proces een stuk lastiger zijn geweest. Jullie vangen de dames liefdevol op en ze zijn gek op jullie.

En dank kom ik uiteindelijk bij mijn gezin… Boy, mijn sharp dressed man! Jij hebt tijdens het hele proces naast me gestaan en ik kan het me niet eens voorstellen hoe deze reis geweest zou zijn zonder jou. Je bent attent, lief, grappig, koppig, maar bovenal mijn rots in de branding. En onze genen gecombineerd zijn gewoon buitenaards goed! Valerie en Lorelei, jullie hebben me laten beseffen wat echt belangrijk is in het leven (en dat was niet altijd mijn PhD). Ik hoopte stiekem op meiden met een sterk karakter, en nu heb ik er twee die leuker, liever, slimmer en eigenwijzer zijn dan ik ooit heb kunnen dromen. Met een heerlijke mix van trots en blijdschap kijk ik uit naar hoe jullie je ontwikkelen tot sterke vrouwen die hun eigen interesses en ambities navolgen. Dit proefschrift draag ik daarom op aan jullie! Hopelijk is dit een voorbeeld dat jullie je eigen pad mogen volgen (ook al duurt het wat langer).