

## ● REVIEW

# Circular RNAs in early brain development and their influence and clinical significance in neuropsychiatric disorders

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## Abstract

Neuropsychiatric disorders represent a set of severe and complex mental illnesses, and the exact etiologies of which are unknown. It has been well documented that impairments in the early development of the brain contribute to the pathogenesis of many neuropsychiatric disorders. Currently, the diagnosis of neuropsychiatric disorders largely relies on subjective cognitive assessment, because there are no widely accepted biochemical or genetic biomarkers for diagnosing mental illness. Circular RNAs (circRNAs) are a novel class of endogenous non-coding RNA (ncRNA) with a closed-loop structure. In recent years, there have been tremendous advances in our understanding of the expression profiles and biological roles of circRNAs. In the brain, circRNAs are particularly enriched and are expressed more abundantly in contrast to linear counterpart transcripts. They are highly active at neuronal synapses. These features make circRNAs uniquely crucial for understanding brain health, disease, and neuropsychiatric disorders. This review focuses on the role of circRNAs in early brain development and other brain-related processes that have been associated with the development of neuropsychiatric disorders. In addition, we discuss the potential for blood or cerebrospinal fluid circRNAs to be used as novel biomarkers in the early diagnosis of neuropsychiatric disorders. The findings reviewed here may provide new insight into the pathological mechanisms underlying the onset and progression of neuropsychiatric disorders.

**Key Words:** autism spectrum disorders; bipolar disorder; brain; exosomal circRNAs; microRNAs; nerve regeneration; non-coding RNAs; obsessive-compulsive disorders; schizophrenia

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## Introduction

Neuropsychiatric disorders are among the leading causes of human disability, resulting in considerable social and economic burdens (GBD 2015 Neurological Disorders Collaborator Group, 2017; Walker and Druss, 2017). Unfortunately, the prevalence rates of most neuropsychiatric disorders, including bipolar disorder, schizophrenia, autism spectrum disorders, and obsessive-compulsive disorders have increased over the past several decades and they continue to rise (GBD 2015 Neurological Disorders Collaborator Group, 2017; Gibbs et al., 2018). The specific etiologies of neuropsychiatric disorders remain unknown. However, the interplay between genetic and environmental factors is thought to contribute to the onset of most neuropsychiatric disorders. Genetic variations are considered as causal factors in contrast to environmental risk factors. Currently, the diagnosis of a specific neuropsychiatric disorder primarily relies on observed disturbances in behavior, including impaired cogni-

tion, perception, and social behavior (American Psychiatric Association, 2013). Despite intense research efforts, there are no objective tests or serum biomarkers for risk assessment, early detection, or early diagnosis, which limits the efficient and timely treatment of patients with neuropsychiatric disorders. There are many other challenges faced by researchers performing neuropsychiatric studies, including the difficulty in obtaining biopsy samples for comparative studies and the lack of suitable animal models that closely mimic abnormal human behaviors.

Circular RNAs (circRNAs) are an emerging class of endogenous non-coding RNA (ncRNA) that contain closed-loop structures and are biosynthesized by a back-splicing event of protein-coding mRNAs that occurs during post-transcriptional processes (Hansen et al., 2013; Hentze and Preiss, 2013; Jeck and Sharpless, 2014; Liu et al., 2019). Recently, considerable research has been performed to assess the biological functions of circRNAs. Results from these studies

show that circRNAs possess regulatory potential as “sponges” for their target microRNAs (miRNAs) and RNA binding proteins. circRNAs are more abundantly expressed in the brain than their linear counterparts, such as miRNAs, and are highly active at neuronal synapses (Jeck et al., 2013; Jeck and Sharpless, 2014; You et al., 2015; Bonizzato et al., 2016; van Rossum et al., 2016; Piwecka et al., 2017). The unique properties of circRNAs in the brain indicate that they may play important roles in maintaining brain health and preventing the development of neurological diseases, including neuropsychiatric disorders.

This review mainly discusses the relationship between circRNAs and early brain development. We also discuss the potential for using blood or cerebrospinal fluid (CSF) circRNAs for the early detection and diagnosis of specific neuropsychiatric disorders, and for delineation of the underlying pathogenic mechanisms. To identify relevant publications within the past 50 years we searched PubMed, Google Scholar, Wanfang Med Online, and Baidu Scholar using the following keywords: circular RNAs, exosome, non-coding RNAs, microRNAs, neuropsychiatric disorders, schizophrenia, bipolar disorder, autism spectrum disorders, and obsessive-compulsive disorders. Among the identified articles, we included the original, most relevant articles and excluded those studies with a lack of relevance.

## Biosynthesis, Function, and Mechanism of Action of circRNAs

circRNAs represent a novel class of naturally occurring ncRNAs that have a single-stranded closed-loop structure and play an essential role in the modulation of gene expression (Salzman et al., 2012; Hansen et al., 2013; Jeck et al., 2013; Memczak et al., 2013; Tay et al., 2014; Qu et al., 2015). They were first detected in the cytoplasm of cells using electron microscopy nearly four decades ago (Hsu and Coca-Prados, 1979). However, circRNAs were thought to be natural by-products with no biological purpose. By the early 1980s, it was shown that circRNAs arose from post-transcriptional splicing and could be associated with the pathogenesis of some diseases (Sanger et al., 1976; Hsu and Coca-Prados, 1979; Arnberg et al., 1980; Nigro et al., 1991; Cocquerelle et al., 1992; Capel et al., 1993). Initially, circRNAs attracted little attention, because there were no technical approaches to investigate their function. With the recent advances in high-throughput sequencing of ribosome RNA (rRNA)-depleted RNA, in combination with bioinformatic tools, research into the biological roles of circRNAs has greatly accelerated in the past decade.

Jeck et al. (2013) performed high-throughput rRNA-depleted RNA sequencing of human fibroblast cells and identified more than 25,000 circRNAs. Unlike linear ncRNAs, such as miRNAs and long non-coding RNAs (lncRNA) that possess 5' and 3' termini, circRNAs lack 5' and 3' ends and are generated during post-transcriptional back-splicing (alternative circularization) of protein-coding regions (exons, introns, or both) via a covalent linkage. The majority of

circRNAs arise from one or more exons of protein-coding genes (Jeck et al., 2013). Consistent with these early findings in human fibroblast cells (Jeck et al., 2013), a recent study in the embryonic brain revealed that a large proportion of circRNAs were aligned to the exons of the human genome, while only a small proportion were transcribed from introns or intergenic regions (Chen et al., 2018). Subsequent studies have found two primary ways for the formation of circRNAs, either via the direct back-splicing of exons or by the alternative splicing of exons. For the back-splicing of exons, intron-pairing of repeated sequences, including *Arthrobacter luteus* (Alu) elements which form highly stable base pairs, is a catalyst to drive and enhance circularization, also referred to as direct back-splicing of exons (Jeck et al., 2013; Liang and Wilusz, 2014; Zhang et al., 2014; Ivanov et al., 2015).

The creation of circRNAs by the alternative splicing of exons, is initiated by the removal of several consecutive exons. Of these mechanisms, the direct back-splicing of exons is considered dominant in the formation and closure of circRNAs. In an effort to understand the mechanisms underlying the biosynthesis of circRNAs, the spliceosome machinery was implicated in the circularization of splicing sites during a synthetic process (Ashwal-Fluss et al., 2014; Starke et al., 2015; Szabo et al., 2015; Wang and Wang, 2015) because the canonical splicing sites are generally needed for circularization. Additionally, Starke et al. (2015) found that isoginkgetin, a specific splicing inhibitor, significantly abrogated the formation of circRNAs. However, it is still unknown how the spliceosome acts to synthesize circRNAs, and how the spliceosome distinguishes between back splicing and linear splicing.

Recently, using a newly developed single-molecule RNA fluorescence *in situ* hybridization (smRNA FISH) approach for two-color imaging, Kocks and colleagues quantified and investigated the subcellular localization of the circRNA, CDR1as, in a P19 neuronal cell line. They demonstrated that CDR1as-positive particles were apparent in cell bodies (soma) and synaptic processes (dendrites and axons) of the P19 neurons (Kocks et al., 2018). These findings are consistent with previous results (You et al., 2015). You et al. (2015) used high-resolution *in situ* hybridization to examine the subcellular localization of circRNAs in hippocampal neurons *in vitro* and in hippocampal slices. They detected circRNAs in both cell bodies and synaptic processes. However, it is still not known how circRNAs are synthesized in neurons and subsequently transported to the cell bodies and synaptic processes.

Gene regulation by circRNAs involves multiple steps and has been demonstrated for a few known circRNAs: (1) circRNAs may interact with their target miRNAs in the cytoplasm to regulate gene expression at the post-transcriptional level. circRNAs can act as “sponges” to competitively absorb miRNAs, thereby lifting the inhibitory effects of miRNAs on their target mRNAs and promoting post-transcriptional gene expression (Hansen et al., 2013; Memczak et al., 2013; Piwecka et al., 2017). (2) circRNAs may function as sponges of RNA-binding proteins to serve as post-transcriptional

regulators of gene expression (Hentze and Preiss, 2013; Jeck and Sharpless, 2014). Dudekula et al. (2015) analyzed large-scale transcriptome data using a combination of bioinformatic tools to establish a powerful online tool for predicting RNA-binding protein-binding sites on circRNAs. (3) circRNAs may exert a regulatory role on gene expression at the transcriptional level (Hansen et al., 2013; Memczak et al., 2013; Zhang et al., 2013; Du et al., 2016).

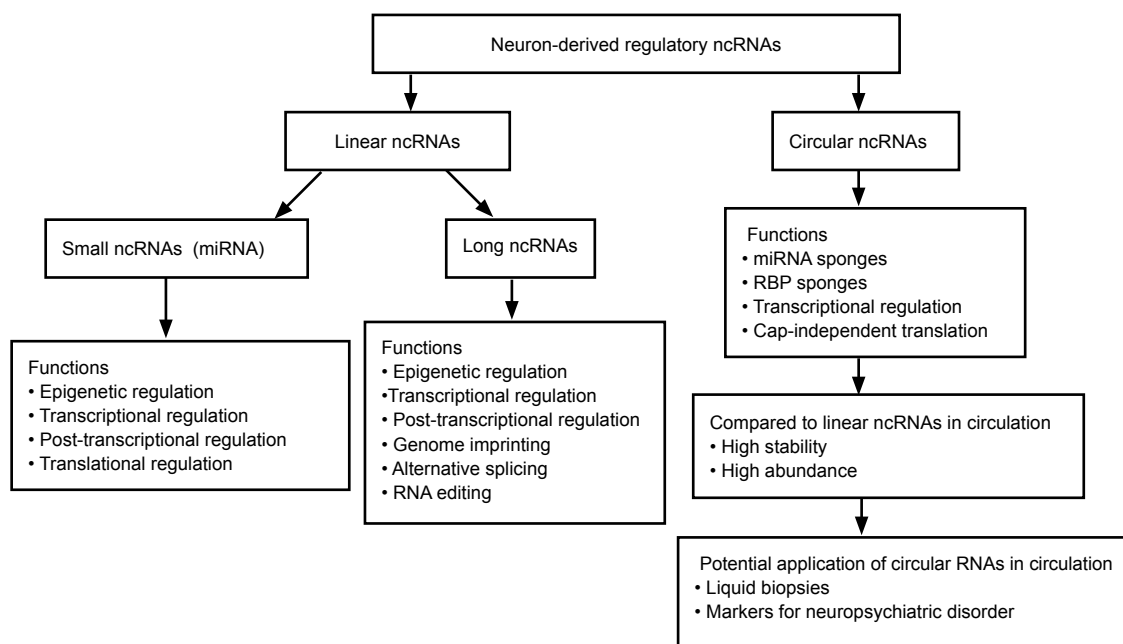
In neurons, the circRNA, Cdr1as, is recognized as a major regulator of some miRNAs. Extensive studies have demonstrated that Cdr1as is involved in the modulation of its target miRNAs (e.g. miR-7, miR-671). The mechanisms of how circRNA Cdr1as interacts with its target linear ncRNAs, and its full functions require further investigation. Recently, Piwecka and colleagues (2017) discovered that Cdr1as in the brain of humans and mice harbors several binding sites for miRNA-7 and miR-671. In a subsequent study, deletion of Cdr1as using CRISPR/Cas9 genome editing technology significantly elevated miR-7 levels and decreased miR-671 levels, indicating direct interactions between Cdr1as and these miRNAs. Notably, the interactions between Cdr1as and its target linear ncRNAs were associated with normal brain function in mice because removal of the Cdr1as locus from the genome caused impairment in sensorimotor gating and deficit in this ability is linked to neuropsychiatric disorders (Piwecka et al., 2017). In addition, other miRNAs remained unaltered in the brain, which is consistent with a previous study (Piwecka et al., 2017). It is important to note that Cdr1as-mediated regulation occurred at the post-transcriptional level in the cytoplasm, and it was postulated that Cdr1as could stabilize miR-7 by serving as a sponge (Piwecka et al., 2017).

Therefore, circRNAs may serve as new players in the tran-

scriptional, post-transcriptional, and translational regulation of genes, while their contributions to human health and disease await further elucidation.

## Expression Patterns of circRNAs and Their Relation to Brain Development and Neurological Processes

Recent years have witnessed tremendous progress in discovering the expression patterns and biological roles of circRNAs in several tissues, including the brain (You et al., 2015; van Rossum et al., 2016; Chen et al., 2018). Neuron-derived linear and circular ncRNAs and their potential functions are summarized in **Figure 1**. Studies performed with both synaptosomes and neuropil dissected from hippocampal slices were consistent with the notion that circRNAs are more abundantly expressed in the brain compared with their linear counterpart transcripts (You et al., 2015; Chen et al., 2018). In a comparative analysis of the abundance and tissue specificity of circRNAs in mice, rRNA-depleted RNA deep sequencing revealed that the relative abundance of circRNA was considerably higher in the brain when compared with other organs, such as the heart, liver, lung, and testis (You et al., 2015). You et al. (2015) compared the abundance ratios for specific circRNAs to the total transcriptional output of the same gene locus and the highest ratio was detected in the brain, in contrast to the other four tissues studied, the heart, liver, lung, and testis. Recently, Chen et al. (2018) characterized circRNAs in different stages of embryonic brain development in humans and found that the abundance and expression profiles of circRNAs changed during different developmental stages of the brain. They detected 4324 and 4425 circRNAs expressed in the early and late stages, respectively, while 6550 circRNAs were expressed across



**Figure 1** Neuron-derived linear and circular non-coding RNA (ncRNAs), as well as their potential functions and proposed applications in neuropsychiatric disorders.

both stages. Of the circRNAs expressed in both stages, 104 were differently expressed between the early and late stages of brain development with the top three being: circRNA SATB homeobox 2, regulating synaptic membrane exocytosis 1, and CTC-525D6.1 (Chen et al., 2018). Interestingly, levels of all three circRNAs were significantly increased in the late stage in comparison with the early stage of brain development with the greatest change (8.21-fold-change) for circRNA SATB homeobox 2 (Chen et al., 2018). In addition, other recent studies have shown that circRNAs are dynamically expressed and temporospatially modulated throughout brain development in mice, rats, and humans (Rybak-Wolf et al., 2015; Veno et al., 2015; Mahmoudi and Cairns, 2019). Notably, the greatest number of developmentally regulated circRNAs has been observed in the brain compared with other studied tissues, including the testes, kidney, thymus, and liver (Mahmoudi and Cairns, 2019). Although the underlying mechanisms of how these developmentally-dependent circRNAs are regulated remain elusive, these findings indicate that these specific circRNAs may play unique roles during the different stages of brain development. It is suggested that circRNAs mainly regulate host gene expression through sponging miRNAs. Mahmoudi and Cairns (2019) investigated circRNA interactions with miRNAs and their target mRNAs, and found that miRNAs could be sponged by circRNAs during brain development in rats, leading to increases in levels of their target mRNAs. The potential regulatory roles or functions of circRNAs are illustrated in **Figure 2**.

Thus, circRNAs possess the following properties: (1) circRNAs are more abundant in the brain compared to other

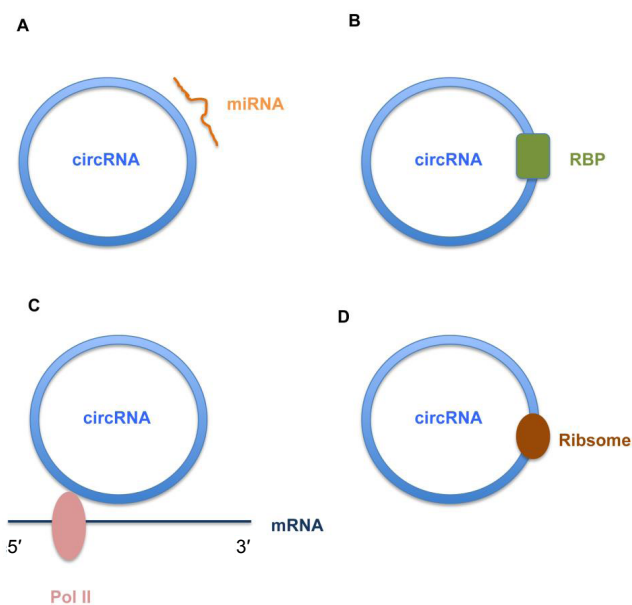
tissues; (2) circRNAs in the brain are particularly enriched at synapses; (3) circRNAs are developmental stage-specific; (4) circRNAs are more stable compared with their linear counterparts; (5) the majority of circRNAs are conserved but circRNAs containing intronic sequences are poorly conserved between species; and (6) circRNAs can be enclosed in exosomes to pass through the blood-brain barrier to enter the circulation.

In two studies in flies and mice, rRNA-depleted RNA deep sequencing revealed that the structural and sequence properties of circRNAs were similar to those found in humans (Westholm et al., 2014; You et al., 2015). In a high-throughput sequencing of the brain synaptosome, You and colleagues (2015) examined circRNAs expression profiles in synapses. It merits attention that the majority of circRNAs were primarily distributed to the synapses, with the most abundant circRNAs in the presynaptic active zone. In addition, they were derived from genes that encoded proteins associated synaptic functions (You et al., 2015). In the same study, You et al. (2015) demonstrated that a large number of circRNAs had altered levels at four different stages of brain development, E18, P1, P10, and P30. Of these, 43 displayed peak expression at E18 and P1, but expression declined during the subsequent developmental stages (You et al., 2015). Interestingly, 181 circRNAs were identified to peak at P10 and P30 (You et al., 2015). The expression of one circRNA, Rmst, declined considerably during the later stages of brain development. Also, levels of circKlhl2 expression increased during the early developmental stages when synapses are formed and matured in mice (You et al., 2015). These findings support of the idea that circRNAs modulate synapse-related functions during neuronal differentiation and brain development.

The brain is the most complex organ with fine-tuned gene expression that ensures the proper functioning of neurological processes, and thus aberrant circRNA expression patterns might be correlated with the development of neuropsychiatric disorders. To determine if these differentially expressed circRNAs are related to the plasticity of synapses, a cell culture model was used to induce plasticity. Interestingly circRNA profiling revealed 37 upregulated circRNAs and five down-regulated circRNAs following the induction of plasticity (You et al., 2015), suggesting a link between these circRNAs and synaptic plasticity. Despite significant progress, our understanding of circRNAs and their relation to brain development is still limited, and their biological roles in neuropsychiatric disorders and in the underlying molecular mechanisms remain to be fully elucidated.

## Exosomal circRNAs in Neuropsychiatric Disorder Research

It is difficult to obtain human brain biopsy specimens for the study of neuropsychiatric disorders, because the human brain is usually inaccessible and tissues are only obtainable through invasive procedures, which are not clinically practical. This is one of the major bottlenecks in brain disease



**Figure 2** Schematic diagram of regulatory roles or functions of circular RNAs (circRNAs).

(A) circRNAs may interact with target miRNAs to regulate gene expression; (B) circRNAs may act as sponges of RNA-binding proteins (RBPs) to regulate gene expression; (C) circRNAs may exert a potential regulatory role through RNA polymerase II (Pol II); (D) circRNAs may be translated into proteins.

research, especially for neuropsychiatric disorders. In addition, few animal models can mimic the clinical manifestations of specific human neuropsychiatric disorders. In fact, researchers have been seeking alternative models that would eliminate the need for human brain biopsies. However, we lack the non-invasive or minimally invasive approaches to determine the genetic changes associated with the pathogenesis of neuropsychiatric disorders. circRNAs are shed from brain cells and enter into the blood and CSF; therefore, liquid biopsies may provide insight into certain circRNAs that originate in the brain.

To date, our knowledge of circulating circRNAs, either in the blood or CSF, and their relationship with neuropsychiatric disorders is scarce. However, extensive studies of circulating ncRNAs, such as miRNAs, lncRNAs, and circRNAs, have been performed in the field of oncology. These studies have paved a road that could be extended to other research areas, including neuropsychiatric disorders. For example, circulating miRNAs in exosomes, which are extracellular vesicles ranging from 40–100 nm in diameter (Xu et al., 2018), that were referred to as exosomal circRNAs (Li et al., 2015), have been extensively investigated in cancer because they maintain the properties from the solid tissues from which they originated (Mitchell et al., 2008; Taylor and Gercel-Taylor, 2008; Xu et al., 2018). Furthermore, the abundance and patterns of exosomal miRNAs differ significantly between patients with cancer and healthy participants, and circulating miRNAs have already been proposed as potential liquid biopsy biomarkers for cancer diagnostics (Skog et al., 2008; Taylor and Gercel-Taylor, 2008; Rabinowits et al., 2009; Lohr et al., 2014; Speicher and Pantel, 2014; Siravegna et al., 2017). These findings are particularly important for the study of brain development and its relationship to psychiatric disorders, considering the unavailability of brain biopsies and the other limitations of studying humans. The use of human blood and CSF to assess circRNA patterns and their relationships with psychiatric disorders may provide valuable information. Thus, the study of exosomal circRNAs in blood or CSF represents a novel approach to deciphering the molecular mechanisms underlying the onset, development, and progression of neuropsychiatric disorders.

There are technical challenges when working with extracellular vesicles, including exosomes. It is difficult to isolate sufficient exosomes from blood or CSF for the analysis of exosomal circRNAs using conventional high-throughput RNA sequencing. This limitation may be overcome with ultra-low input RNA sequencing and the development of novel approaches for exosome preparation. Im et al. (2014, 2015) developed a novel technique for exosome isolation based on surface plasmon resonance, in which a chip called a nano-plasmonic exosome sensor, allows for the specific capture of exosomes with optimal accuracy. Notably, the nano-plasmonic exosome chip only requires a small quantity of sample and the system displays high sensitivity and specificity, which is beneficial for the preparation of exosomes from low-volume CSF samples.

## Perspectives on Exosomal circRNAs as Potential Biomarkers for the Early Detection and Diagnosis of Neuropsychiatric Disorders

The development of novel non-invasive serum biomarkers for the early detection of neuropsychiatric disorders is among the top priorities for clinical neuropsychiatrists. Interestingly, circRNAs are particularly abundant and highly active in synaptoneurosomes when compared with the cytoplasm and whole-brain lysates (Rybak-Wolf et al., 2015; Lu and Xu, 2016). Given that synaptic dysfunction has been closely linked to abnormalities in early brain development and synaptic plasticity of many neuropsychiatric disorders, these findings indicate that aberrant expression of circulating circRNAs associated with synaptoneurosomes has the potential to provide potential biomarkers for the early detection and diagnosis of neuropsychiatric disorders. Recently, Xu et al. (2018) found that many circRNAs exist abundantly and stably in exosomes or extracellular vesicles released by most mammalian cells. These are also referred to as exosomal circRNAs (Li et al., 2015). In the same study, Li et al. (2015) characterized profiles of exosomal circRNAs in human blood samples obtained from healthy individuals by deep RNA sequencing and identified 1215 circRNAs < 1000 nt in length in exosomes. Of these, the majority (> 90%) were generated from protein-coding exons, with a small proportion derived from introns or other regions of the human genome, which agrees with the characteristic origin of circRNAs. Like other forms of ncRNAs, the levels of exosomal circRNAs in the blood were detectable and quantifiable via quantitative reverse transcription-polymerase chain reaction analysis. In addition, a small set of cancer-related exosomal circRNAs was identified, indicating the potential use of circulating exosomal circRNAs as biomarkers for cancer diagnostics (Li et al., 2015).

The inward budding of endosomal membranes forms exosomes, and recent studies have shown that neurons can secrete exosomes containing various ncRNAs, such as miRNAs and lncRNAs (Budnik et al., 2016). Exosomes with an average size of less than 100 nm in diameter can traverse the blood-brain barrier to enter into the blood and CSF, suggesting the circRNAs can actively move out of the brain (Faure et al., 2006; Janas et al., 2016; Lim and Lee, 2017). This supports the idea that exosomal circRNAs may be excellent biomarkers for the early detection of neuropsychiatric disorders. Exosomal miRNAs have also been identified in CSF samples, and an analysis of circulating miRNAs in CSF has been proposed for the diagnosis of primary central nervous system lymphoma (Baraniskin et al., 2011). Additionally, specific exosomal miRNAs have been used as noninvasive biomarkers for monitoring the therapeutic response in patients receiving treatment for glioblastoma (Baraniskin et al., 2011; Samuel et al., 2014; Saadatpour et al., 2016; Siravegna et al., 2017).

Therefore, exosomal circRNAs may represent a better type of biomarker for diagnosing neuropsychiatric disorders in humans because of the following four properties: (1) exo-

somal circRNAs are highly stable, mainly because of their structural features of not having 3' and 5' ends and their location inside exosomes, which protect them from degradation by RNAases; (2) exosomal circRNAs are shed from brain cells to enter the blood and CSF while maintaining the brain expression patterns of circRNAs (origination features); (3) exosomal circRNAs can be detected in exosomes that come from the blood and CSF via reverse transcription-polymerase chain reaction; and (4) exosomal circRNAs specifically associated with variable phenotypes of psychiatric disorders can overcome the limitations of conventional diagnostic modalities currently used to diagnose neuropsychiatric disorders.

## Conclusions

circRNAs are developmental stage-specific in the human brain and are particularly abundant and highly active at the synapses of neurons. The circRNAs associated with early brain development are important for understanding the pathological mechanisms underlying neuropsychiatric disorders. Moreover, neuropsychiatric disorder-associated circRNAs in the blood and CSF hold promise as potential non-invasive biomarkers for the early detection and diagnosis of complex neuropsychiatric disorders.

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