Dysregulation of circRNA Expression in the Peripheral Blood of Individuals With Schizophrenia and Bipolar Disorder

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Abstract

Circular RNA (circRNA) are head-to-tail back-spliced RNA transcripts that have been linked to several biological processes and their perturbation is evident in human diseases, including neurological disorders. There is also emerging research suggesting circRNA expression may also be altered in psychiatric and behavioural syndromes. Here, we provide a comprehensive analysis of circRNA expression in peripheral blood mononuclear cells (PBMCs) from 39 patients with schizophrenia and bipolar disorder as well as 20 healthy individuals using deep RNA-seq. We observed systematic alternative splicing leading to a complex and diverse profile of RNA transcripts including 8,762 high confidence circRNAs. More specific scrutiny of the circular transcriptome in schizophrenia and bipolar disorder, compared to a non-psychiatric control group, revealed significant dysregulation of 55 circRNAs with a bias towards downregulation. These molecules were predicted to interact with a large number of miRNAs that target genes enriched in psychiatric disorders. Further replication and cross validation to determine the specificity of these circRNAs across broader diagnostic groups and subgroups in psychiatry will enable their potential utility as biomarkers to be established.

Introduction

Schizophrenia (SZ) and bipolar disorder (BD) are complex neuropsychiatric conditions with a range of severe symptoms, usually emerging in adolescence and early adulthood, such as delusion, hallucination, cognitive deficit, depression and mania [1,2]. While they have unique aspects, SZ and BD share common clinical features, genetic determinants and treatment response. Evidence from family, twin and adoption studies have supported that both SZ and BD exhibit strong genetic components and high heritability at 60%-80%, with overlapping genetic risk and gene expression profiles [3,4]. Genetic and epigenetic changes that affect RNA level in particular, can play a substantial role in the etiological pathways by directly modifying the mRNA template or by altering posttranscriptional regulation of gene expression. Non-coding RNA including small microRNA and longer lncRNA play an important part in regulating gene expression and appear to be altered by the genetic and environmental exposures associated with psychotic disorders, suggesting they may be essential for the normal physiology and homeostasis of the nervous system [5-7].

While the biological significance of lncRNA is now well established, a novel subclass of covalently closed circular lncRNA or circRNA has also emerged [8]. This conserved family of RNA transcripts is generated by an alternative splicing mechanism, known as back-splicing, in which a donor splice site is joined to an acceptor splice site. These molecules represent several specific features that make them distinguishable in mammalian cells. CircRNAs can be spliced from a combination of host exons resulting in the production of different variants from a single gene [9]. The expression profile of the circRNA have demonstrated a cell type-, tissue- and stage-specific pattern [10-12]. These molecules can function as competitive endogenous RNAs (ceRNAs) by sponging miRNA molecules to control their accessibility, thus indirectly regulating their target's expression [13,14]. They can also control gene transcription by associating with transcription complex [15]. Their ability to sequester RNA-binding proteins (RBPs) and

forming RNA-protein complexes allow circRNA to be involved in intracellular localization and transport of RBPs and associated mRNAs [16,17]. More recently, some circRNAs were shown to be capable of translating into functional proteins [18]. There is growing evidence suggesting circRNA involvement in the pathogenesis of human diseases, such as autoimmunity [19], cancer [20], cardiovascular diseases [21], as well as CNS disorders including SZ, major depressive disorder (MDD), multiple sclerosis (MS), and Alzheimer's disease (AD) [22]. Recent studies have shown perturbation of these molecules in human fluids including peripheral blood, and suggest that some circRNAs might be potential candidates for prediction of disease states or treatment responses [23]. For example, 406 circRNAs were dysregulated in peripheral blood of multiple sclerosis patients with two displaying predictive validity [24]. Similarly, a global alteration of circRNAs was reported in amyotrophic lateral sclerosis (ALS), among which hsa_circ_0023919, hsa_circ_0063411, and hsa_circ_0088036 were supported as potential biomarkers [25]. CircRNA_103636 expression was associated with successful antidepressant treatment in blood of individuals with MDD and therefore maybe predictive of treatment response [26].

CircRNA has also been implicated in the regulation of neuronal activity and plasticity, synaptic transmission, and neuronal depolarization, which are all critical processes in pathophysiology of psychiatric disorders [27-29]. While circRNA appear to be dysregulated in the brain, the genetic and environmental exposures that give rise to this may also manifest in peripheral tissues, such as blood. As these cells are highly accessible through liquid biopsy, their circRNA profile could provide useful insights into aspects of the molecular pathology of psychiatric conditions that could guide further advances related to their diagnosis and treatment. In this study, we provide a comprehensive analysis of circRNA expression patterns and characteristics in the PBMCs of SZ and BD, as well as in healthy controls (HC), and examine their dysregulation in these psychotic disorders.

Materials And Methods

Sample collection

Participants provided signed informed consent. This study was approved by the Human Research Ethics committees of the University of New South Wales (HC12384), St Vincent's Hospital (HREC/10/SVH/9) and the South East Sydney and Illawarra Area Health Service (HREC 09/081). PBMCs were collected from 20 participants with schizophrenia, 19 with bipolar disorder, and 20 non-psychiatric controls following the application of exclusion criteria and QC processes. For additional details, see Supplementary Information.

RNA extraction, Library preparation and RNA-seq

The PBMC samples were RNA extracted, quality controlled, and 300ng RNA was subjected to rRNA depletion followed by fragmentation and library preparation before sequencing with 200 cycles of paired-end. For additional details, see Supplementary Information.

CircRNA detection

Sequencing reads were quality checked and subjected to CIRIquant pipeline [30] to predict circRNA species. A total of 108,865 circRNAs were initially identified in all samples, however in order to obtain high confidence circRNAs we used a filtering cut-off minimum of two junction reads in at least five samples, which allowed a minimum of 10 back-splice junction reads (BSJ) per circRNA. This criterion resulted in 8,762 unique circRNAs in all samples, and we used these high confidence circRNAs for all the analyses performed in this study.

Differential expression analysis

Differential expression of circRNA was performed using DE pipeline in CIRIquant that implements a modified version of edgeR [31] fed with BSJ reads and total mapped reads. We used sex and age as covariates. To detect significant differences in the ratio of circRNA to linear RNA between the conditions, we used a linear model adjusted for sex and age using the Voom package [32]. For additional details, see Supplementary Information.

Detection of circular to linear RNAs ratio(CLR)

We used CIRIquant [30] to obtain the circular to linear transcribes ratio using the FSJs and BSJs, detected by CIRI2, and the following formula: $2 \times \text{bsj} / (2 \times \text{bsj} + \text{fsj})$. For additional details, see Supplementary Information.

cDNA synthesis and quantitative PCR

The Total/RNase R-treated RNA was reverse transcribed with random hexamers. Real-time PCR was performed using divergent primers. For additional details, see Supplementary Information. We used ΔCt method to normalize the data using GAPDH and HMBS as internal references. The Student's t-test (one-tailed) was used to compare expression levels. Primer sequences are provided in Supplementary Table S1.

Detection of alternative splicing

We applied CIRI-AS pipeline [33] to identify alternative splicing (AS) events using paired-end reads. This approach employs a *de novo* assembly algorithm which is able to specifically detect circRNA exons and AS events. For additional details, see Supplementary Information.

Prediction of circRNA interactions

Sequence of the circRNAs were obtained from the circAtlas 2.0 database [34]. Then, miRNA binding sites were predicted using miRanda [35] and TargtScan [36], and circRNAs predicted by both tools were used for circRNA-miRNA interaction analysis. The potential associations of the circRNA and RBP were determined by the "network" tool from circAtlas [34]. miRNA target gene prediction and enrichment analysis are detailed in Supplementary Information.

Protein coding potential, modification and disease involvement

To analyse the protein coding features of the circRNAs we used circbank database [37]. The same database was also used to identify the m6A modification within the circRNA transcripts. We also used circRNA-disease databases circ2disease [38], circad [39], circAtlas [34] and circRNADisease [40] to intersect our differentially expressed circRNAs to those reported in previous studies.

Cellular localization prediction and functional enrichment

To predict cellular localization of all the identified circRNAs we used CSCD database [41]. Overrepresentation analysis was conducted using Gene Ontology (GO) annotation [42,43]. Pathway overrepresentation analysis was additionally run using PANTHER pathway database version 15.0 [44]. For additional details, see Supplementary Information.

Visualization

All the plots and graphs were generated in R. Venn diagrams were produced with the VennDiagram package [45]. Heatmaps were constructed using the heatmap.2 function from the gplots package (<http://CRAN.R-project.org/package=gplots>). The remaining plots and graphs were generated with ggplot2 package [46].

Results

CircRNA are differentially expressed in psychiatric disorders

We identified a total of 8,762 high confidence circRNAs in all the 59 samples (Supplementary Table S2). To explore the implication of PBMC circRNA in the psychiatric disorders we performed differential expression in SZ and BD versus HC individuals. To control for the potentially confounding impacts of sex and age, these factors were included as covariates in our model. We also restricted analyses to the broadly expressed circRNAs, as defined by those circRNAs that were expressed in at least 10 samples for each comparison analysis (SZ vs HC and BD vs HC), to ensure the reliability of findings. A total of 22 and 33 circRNAs were found to be significantly changed in SZ and BD, respectively, as compared to the healthy control group ($P < 0.05$, $FC > 2$) (Fig. 1A; Supplementary Table S3). Notably, 71% of these circRNAs were downregulated, including 14 circRNAs in SZ and 26 in BD group. There were two circRNAs, circNCF1 and circLINC00969 common between the SZ and BD differential expression lists. Annotation of the DE circRNAs showed that 49 circRNAs are exonic, 2 intronic and 2 intergenic circRNAs. Also, 45 of the DE circRNAs are derived from protein coding genes, while 2 and 3 are from lincRNA and pseudogenes, respectively. In addition to the conventional case control analysis, we also combined the psychosis groups and compared to controls and observed three differentially expressed circRNAs including circUGP2, circNCF1C and circKLHDC4 ($P < 0.05$, $FC > 2$) (Supplementary Table S3). In addition, we compared the circRNA expression between the psychosis groups (SZ vs BD) and observed six differentially expressed circRNAs including four that were upregulated and two downregulated in SZ, one of which, circNCF1, was also differentially expressed in the SZ/BD vs HC groups analyses (Supplementary Table S3).

We next determined whether the ratio of circRNA over linear RNA is changed in psychiatric conditions. A linear model including sex and age as covariates was used for the circRNA subset used in DE analysis. The analysis showed there was 33 circRNAs with ratios significantly changed in SZ, compared to the healthy controls ($P < 0.05$, $FC > 1.5$) (Fig. 1B; Supplementary Table S3). However, no significant alteration of the ratio was observed in BD group.

To validate the results of circRNA sequencing, we randomly selected eight significantly dysregulated circRNAs in SZ and BD and conducted qPCR using PBMCs from 21 SZ and 21 BD patients compared to 21 sex- and age-matched healthy controls to analyse the expression levels. Our data confirmed that the tested circRNAs were differentially expressed in psychiatric patients compared to healthy individuals, indicating that the sequencing results are reliable (Fig. 2).

Functional significance of dysregulated circRNAs

CircRNA have emerged as active molecules with biological significance that contribute to the key regulatory programs through interacting with miRNA and RNA binding proteins (RBP), or by translating into peptides [16,17]. In order to gather insight into potential function of the dysregulated circRNAs, we investigated their association with miRNA and RBP, and also their potential for hosting translation elements. We found 45 circRNAs contain at least two binding sites for miRNA which was supported by two prediction tools, miRanda [35] and TargetScan [36] that suggest these molecules could function as competing endogenous RNA for the target mRNA. Collectively we identified 351 circRNA-miRNA interactions, including 148 unique miRNAs (Supplementary Table S4). Interestingly, we found targets of these miRNA were significantly enriched in neurodevelopmental and neurobehavioral disorders including SZ, BD, autism, and mental retardation. GO analysis of circRNA interacting miRNA target genes showed an enrichment of neuronal terms such as neurogenesis, synapse, and neuronal differentiation and development (Supplementary Table S4). We also detected RBP sites within all the circRNAs, with a minimum of two to hundreds of binding sites for each circRNA (Supplementary Table S4). The most frequent circRNA binding factors were ESR1, TRPS1 and BRD4. While our analysis predicted six circRNAs have the potential to translate into proteins (coding probability > 0.9) with open reading frames between ~300-4,000 bp (Supplementary Table S4), we also identified 20 differentially regulated circRNA that have been observed to be m⁶A modified [47]. This is potentially functionally significant given that m⁶A modification of circRNAs has been suggested to enhance the initiation of protein translation [47] (Supplementary Table S4). These analyses suggest that the observed alteration in circRNAs could have functional significance in the PBMCs, which may contribute to the psychopathological conditions.

Alternative splicing profiles in diagnosis groups

We investigated the internal structure of all the identified circRNA by looking at the alternative patterns of splicing of circRNA exons in SZ, BD and HC groups. Our results revealed a total of 2,658 AS events occurred in all the groups (883 unique events) (Supplementary Table S5). We found 545 AS events in the disease groups that were not present in the healthy controls, of which 150 events were common between

SZ and BD. There were also 120 events exclusively occurred in the HC (Fig. 3A). However, the composition of the AS events showed a similar pattern across the groups, with the A3SS showed the highest and IR the lowest fractions (Fig. 3B). Next, we analysed the relative abundance of the events across the studied groups using “percentage spliced in” (PSI) values calculated by the CIRI-AS [33], and the heatmap results showed the top 26 AS circexons were broadly expressed across the diagnosis groups, while the remaining AS circexons were sporadically present in certain samples (Fig. 3C). Differential analysis using PSI showed four and three AS events were significantly altered in SZ and BD, respectively, as compared to HC ($P < 0.05$) (Supplementary Table S5). Collectively, these results suggested distinct circular alternative splicing patterns in the diagnosis groups and also their potential function in regulatory pathways in PBMCs.

Validation and characterization of the identified circRNA

We compared all the identified circRNA with ~140,000 circRNAs in circBase [48], and also with ~100,000 circRNAs detected in our previous studies [49,29] and found an additional 754 circRNAs not previously annotated in either database. To validate the authenticity of the identified circRNAs, we randomly selected 15 circRNAs with different expression levels, mostly from the newly identified candidates (circKLRC4, circFAM107B, circSKAP1, circUTRN, circATP8B4, circPAK2, circABCA13, and circTMEM154), and performed qPCR using specific divergent primers (Supplementary Table S1). The results validated all the 15 tested circRNAs that showed 10- to 50-fold more resistance compared to the linear transcripts, following the RNase R treatment (Fig. 4A). In order to glean insight into the possible biological significance of all the detected circRNAs, we performed pathway overrepresentation analysis for their parental genes and the results indicated a significant enrichment of pathways that are relevant to immune system such as *B cell activation*, *T cell activation*, and *interleukin signalling* (Fig. 4B). The circRNAs were predicated to colocalized in multiple cellular locations, with more circRNAs observed in cytosolic (7,162) followed by nuclear and membrane fractions, in contrast to chromatin that contain the least number of circRNAs (1,248) (Supplementary Table S6). Analysis of the back-splicing frequency showed 167 circRNAs with a circular to linear reads (CLR) > 0.66 , indicating these circRNAs had higher expression levels than their linear counterparts. (Supplementary Table S6). As shown in the heatmap plot (Fig. 4C), most of these circRNAs showed high CLR across the studied groups, suggesting their potential biological significance in gene expression regulation.

Discussion

CircRNA are highly conserved molecules with distinct and specific expression pattern across tissues and species [50,51]. Their covalently closed ends also endow these molecules with high resistance to degradation such that their half-life over 48h is much longer than comparative linear RNA [52]. These special characteristics make circRNA favourable candidates as biomarkers for human disease, particularly in relation to disorders where access to tissues is limited and liquid biopsy from circulating tissue or fluids such as blood, exosomes and extracellular vesicles [53-57] is preferable. In support of this approach, we observed 22 and 33 circRNAs that were significantly altered in circulating PBMCs from

individuals with SZ and BD, respectively, compared to non-psychiatric controls, three of which were also observed to be altered in the combined psychosis group when compared with controls. Most of the differentially expressed circRNAs were downregulated including two (circNCF1 and circLINC00969) that were shared between both disorders. One of these molecules, circDPYD, was recently shown to be dysregulated in plasma samples from coronary artery disease (CAD) patients and was suggested to regulate TRPM3 through the suppression of miR-130a-3p [58]. Interestingly, the circRNA arises from DPYD, a gene located at the genome-wide significant risk loci for schizophrenia [59]. Another circRNA, circARHGEF12, was also reported to be significantly downregulated in gastric cancer tissues compared to the paired nontumorous tissue [60]. It is worth to note that we crossed referenced the altered circRNAs to those expressed in human brain and found the majority of altered circRNAs (48 out of 53) are expressed in the brain. In previous work in psychiatry Yao *et al.* observed 9 differentially expressed circRNAs in PBMCs from a small sample of individuals with SZ (n=9) and non-psychiatric controls, using microarrays [61]. While this finding was supported in a larger validation sample by qPCR we could not replicate their results in our cohort, that may reflect a heterogeneity between the two cohorts, which is particularly noticeable in the different sample sizes. Also, different expression profiling platforms may contribute to the observed inconsistency.

The circRNA interaction analysis of differentially expressed circRNAs suggested they modulate a diverse profile of target miRNA in PBMCs. Six of the interacting miRNA including miR-564, miR-572, miR-107, miR-1275, miR-576-5p, hsa-miR-342-5p and miR-330-3p were previously reported to be dysregulated in the peripheral blood of patients with SZ and/or BD [62-65]. Our analysis of gene targets of the interacting miRNA revealed a substantial enrichment of the targets in brain disorders such as SZ and BD, also in GO neuronal clusters including synapse, axon guidance and neurogenesis. These data suggest that the dysregulated circRNAs might play an intermediate regulatory role in pathogenesis of these disorders by modulating the miRNA levels. Nevertheless, due to limitations of the in silico analyses such as prediction errors and lack of simulation of real biological environment, experimental studies are warranted to validate these bioinformatic-based findings. Association of circRNA with miRNA was shown to be critical for normal physiology, and the perturbation of this interaction may lead to a pathological condition. For example, dysregulated CDR1as altered miR-7 accessibility and its target genes, resulted in deficits in synaptic neurotransmission and abnormal brain function associated with psychiatric disorders [28]. circHomer1a, a neuronal-enriched circRNA associated with neural plasticity [27], is decreased in the prefrontal cortex (PFC) and stem cell-derived neuronal cultures from patients with SZ and BD [66]. Furthermore, circHomer1a expression levels in dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) were positively associated with the age of onset of schizophrenia [66]. Functional role of circMTO1 was initially reported in Hepatocellular carcinoma (HCC), where suppression of HCC progression was regulated by circMTO1 acting as the sponge of oncogenic miR-9 to promote p21 expression, suggesting its potential as a HCC treatment target [67]. Following studies indicated circMTO1 roles in various cancer pathways through inhibiting its target miRNAs including miR-9, miR-223, miR-630, miR-92 [68,69]. These evidences confirm the functional association of the circRNA transcripts in the pathogenesis of human diseases, mostly through regulating miRNA accessibility.

We also detected RBP sites within the changed circRNAs, with some circRNAs containing more than a hundred binding sites for the interacting proteins. Interaction with RBP may contribute to the transportation, preservation or transcription ability of the target proteins [16,17]. It has been recently shown that circRNAs are capable of producing functional proteins [18]. As such, we assessed the translation features in our altered circRNAs and found six which have the potential to encode a protein with open reading frame above 300 bp. Interestingly, more than a third of our list of dysregulated molecules were among circRNAs previously shown to be the subject of m⁶A modification, which is thought to be associated with circRNA translation [47].

Our analyses, along with current evidence, suggests that circRNAs could have functional significance in the PBMCs, through which they could associate with neuropathological features. This is due to the immunological link to the pathophysiology of SZ and BD supported by several genome-wide association studies and gene expression reports that showed pathways involved in immunity, apoptosis and inflammation are significantly enriched in these disorders [65]. In addition, maternal infection and birth in the peak infection seasons were indicated to have a critical role in SZ and associated cognitive deficits through impairing the immune functions [65]. These lines of evidence support immunological manifestation of the disorders, suggesting that PBMCs reflect genomic alterations observed in patients with psychiatric disorders; and thus they can be insightful in investigations of the etiology of these diseases.

Furthermore, we observed some circRNAs have a higher relative back-splicing frequency compared with their linear counterparts, suggesting their potential biological function in regulation of gene expression. Our investigation of potential exon usage within PBMC circRNA structures revealed a diverse array of alternative splicing involved in circRNA biogenesis, with a bias towards the A3SS event. While some AS events were shared between the diagnosis groups, some were exclusively expressed in one group. We also found several AS events were significantly altered in SZ and BD, as compared to HC. These data suggest that alternative splicing in circRNA might be essential for PBMCs physiology, and the splicing pattern of some circRNAs could have important pathological significance.

In summary, we characterized circRNA transcript expression in human PBMCs from SZ and BD patients and observed changes compared to non-psychiatric controls. Further bioinformatic analyses of psychosis associated circRNA, showed they may function as ceRNA for mRNA and form a miRNA interacting platform with the potential to modulate genes enriched in psychiatric conditions. While these preliminary findings are tantalizing, large-scale replication and validation are also warranted to confirm their biological role and potential application as biomarkers with relevance to psychiatric disease and treatment.

Declarations

Funding

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Conflict of Interest

The authors declare no financial interests or potential conflicts of interest.

Ethics approval

This study was approved by the Human Research Ethics committees of the University of New South Wales (HC12384), St Vincent's Hospital (HREC/10/SVH/9) and the South East Sydney and Illawarra Area Health Service (HREC 09/081).

Consent to participate

Participants provided signed informed consent.

Consent for publication

Not Applicable.

Availability of data and material

As the sequencing data belong to patients we can not be made them available at this stage.

Code availability

Not applicable

Authors' contributions

EM was involved in the research design, methodology and data analysis, and writing the first draft. MG contributed to the study design, data interpretation, and revising the manuscript. MC was involved in the supervision of the project, design of the study, interpretation of data, and revising the manuscript. All authors read and approved its final version.

References

1. Patel KR, Cherian J, Gohil K, Atkinson D (2014) Schizophrenia: overview and treatment options. *P T* 39 (9):638-645
2. Vieta E, Berk M, Schulze TG, Carvalho AF, Suppes T, Calabrese JR, Gao K, Miskowiak KW, Grande I (2018) Bipolar disorders. *Nat Rev Dis Primers* 4:18008. doi:10.1038/nrdp.2018.8

3. Cardno AG, Owen MJ (2014) Genetic relationships between schizophrenia, bipolar disorder, and schizoaffective disorder. *Schizophr Bull* 40 (3):504-515. doi:10.1093/schbul/sbu016
4. Shao L, Vawter MP (2008) Shared gene expression alterations in schizophrenia and bipolar disorder. *Biol Psychiatry* 64 (2):89-97. doi:10.1016/j.biopsych.2007.11.010
5. Geaghan M, Cairns MJ (2015) MicroRNA and Posttranscriptional Dysregulation in Psychiatry. *Biol Psychiatry* 78 (4):231-239. doi:10.1016/j.biopsych.2014.12.009
6. Mahmoudi E, Cairns MJ (2017) MiR-137: an important player in neural development and neoplastic transformation. *Mol Psychiatry* 22 (1):44-55. doi:10.1038/mp.2016.150
7. Rusconi F, Battaglioli E, Venturin M (2020) Psychiatric Disorders and lncRNAs: A Synaptic Match. *Int J Mol Sci* 21 (9). doi:10.3390/ijms21093030
8. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495 (7441):333-338. doi:10.1038/nature11928
9. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, Zhang J, Chen LL, Yang L (2016) Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. *Genome Res* 26 (9):1277-1287. doi:10.1101/gr.202895.115
10. Mahmoudi E, Cairns MJ (2019) Circular RNAs are temporospatially regulated throughout development and ageing in the rat. *Sci Rep* 9 (1):2564. doi:10.1038/s41598-019-38860-9
11. Gaffo E, Boldrin E, Dal Molin A, Bresolin S, Bonizzato A, Trentin L, Frasson C, Debatin K-M, Meyer LH, te Kronnie G, Bortoluzzi S (2019) Circular RNA differential expression in blood cell populations and exploration of circRNA deregulation in pediatric acute lymphoblastic leukemia. *Scientific Reports* 9 (1):14670. doi:10.1038/s41598-019-50864-z
12. Veno MT, Hansen TB, Veno ST, Clausen BH, Grebing M, Finsen B, Holm IE, Kjems J (2015) Spatio-temporal regulation of circular RNA expression during porcine embryonic brain development. *Genome Biol* 16:245. doi:10.1186/s13059-015-0801-3
13. Zhong Z, Lv M, Chen J (2016) Screening differential circular RNA expression profiles reveals the regulatory role of circTCF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma. *Sci Rep* 6:30919. doi:10.1038/srep30919
14. Liu Q, Zhang X, Hu X, Dai L, Fu X, Zhang J, Ao Y (2016) Circular RNA Related to the Chondrocyte ECM Regulates MMP13 Expression by Functioning as a MiR-136 'Sponge' in Human Cartilage Degradation. *Sci Rep* 6:22572. doi:10.1038/srep22572
15. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G (2015) Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* 22 (3):256-264. doi:10.1038/nsmb.2959
16. Haque S, Harries LW (2017) Circular RNAs (circRNAs) in Health and Disease. *Genes (Basel)* 8 (12). doi:10.3390/genes8120353

17. Fischer JW, Leung AK (2017) CircRNAs: a regulator of cellular stress. *Crit Rev Biochem Mol Biol* 52 (2):220-233. doi:10.1080/10409238.2016.1276882
18. Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, Fatica A, Santini T, Andronache A, Wade M, Laneve P, Rajewsky N, Bozzoni I (2017) Circ-ZNF609 Is a Circular RNA that Can Be Translated and Functions in Myogenesis. *Mol Cell* 66 (1):22-37 e29. doi:10.1016/j.molcel.2017.02.017
19. Xia X, Tang X, Wang S (2019) Roles of CircRNAs in Autoimmune Diseases. *Front Immunol* 10:639. doi:10.3389/fimmu.2019.00639
20. Su M, Xiao Y, Ma J, Tang Y, Tian B, Zhang Y, Li X, Wu Z, Yang D, Zhou Y, Wang H, Liao Q, Wang W (2019) Circular RNAs in Cancer: emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers. *Molecular Cancer* 18 (1):90. doi:10.1186/s12943-019-1002-6
21. Fan X, Weng X, Zhao Y, Chen W, Gan T, Xu D (2017) Circular RNAs in Cardiovascular Disease: An Overview. *Biomed Res Int* 2017:5135781. doi:10.1155/2017/5135781
22. Mehta SL, Dempsey RJ, Vemuganti R (2020) Role of circular RNAs in brain development and CNS diseases. *Prog Neurobiol* 186:101746. doi:10.1016/j.pneurobio.2020.101746
23. Lei B, Tian Z, Fan W, Ni B (2019) Circular RNA: a novel biomarker and therapeutic target for human cancers. *Int J Med Sci* 16 (2):292-301. doi:10.7150/ijms.28047
24. Iparraguirre L, Munoz-Culla M, Prada-Luengo I, Castillo-Trivino T, Olascoaga J, Otaegui D (2017) Circular RNA profiling reveals that circular RNAs from ANXA2 can be used as new biomarkers for multiple sclerosis. *Hum Mol Genet* 26 (18):3564-3572. doi:10.1093/hmg/ddx243
25. Dolinar A, Koritnik B, Glavač D, Ravnik-Glavač M (2019) Circular RNAs as Potential Blood Biomarkers in Amyotrophic Lateral Sclerosis. *Molecular Neurobiology* 56 (12):8052-8062. doi:10.1007/s12035-019-1627-x
26. Cui X, Niu W, Kong L, He M, Jiang K, Chen S, Zhong A, Li W, Lu J, Zhang L (2016) hsa_circRNA_103636: potential novel diagnostic and therapeutic biomarker in Major depressive disorder. *Biomark Med* 10 (9):943-952. doi:10.2217/bmm-2016-0130
27. You X, Vlatkovic I, Babic A, Will T, Epstein I, Tushev G, Akbalik G, Wang M, Glock C, Quedenau C, Wang X, Hou J, Liu H, Sun W, Sambandan S, Chen T, Schuman EM, Chen W (2015) Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci* 18 (4):603-610. doi:10.1038/nn.3975
28. Piwecka M, Glazar P, Hernandez-Miranda LR, Memczak S, Wolf SA, Rybak-Wolf A, Filipchuk A, Klironomos F, Cerda Jara CA, Fenske P, Trimbuch T, Zywitzka V, Plass M, Schreyer L, Ayoub S, Kocks C, Kuhn R, Rosenmund C, Birchmeier C, Rajewsky N (2017) Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. *Science* 357 (6357). doi:10.1126/science.aam8526
29. Mahmoudi E, Kiltschewskij D, Fitzsimmons C, Cairns MJ (2019) Depolarization-Associated CircRNA Regulate Neural Gene Expression and in Some Cases May Function as Templates for Translation. *Cells* 9 (1). doi:10.3390/cells9010025

30. Zhang J, Chen S, Yang J, Zhao F (2020) Accurate quantification of circular RNAs identifies extensive circular isoform switching events. *Nat Commun* 11 (1):90. doi:10.1038/s41467-019-13840-9
31. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26 (1):139-140. doi:10.1093/bioinformatics/btp616
32. Law CW, Chen Y, Shi W, Smyth GK (2014) voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol* 15 (2):R29. doi:10.1186/gb-2014-15-2-r29
33. Gao Y, Wang J, Zheng Y, Zhang J, Chen S, Zhao F (2016) Comprehensive identification of internal structure and alternative splicing events in circular RNAs. *Nat Commun* 7:12060. doi:10.1038/ncomms12060
34. Wu W, Ji P, Zhao F (2020) CircAtlas: an integrated resource of one million highly accurate circular RNAs from 1070 vertebrate transcriptomes. *Genome Biology* 21 (1):101. doi:10.1186/s13059-020-02018-y
35. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS (2003) MicroRNA targets in *Drosophila*. *Genome Biol* 5 (1):R1. doi:10.1186/gb-2003-5-1-r1
36. Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19 (1):92-105. doi:10.1101/gr.082701.108
37. Liu M, Wang Q, Shen J, Yang BB, Ding X (2019) Circbank: a comprehensive database for circRNA with standard nomenclature. *RNA Biol* 16 (7):899-905. doi:10.1080/15476286.2019.1600395
38. Yao D, Zhang L, Zheng M, Sun X, Lu Y, Liu P (2018) Circ2Disease: a manually curated database of experimentally validated circRNAs in human disease. *Scientific Reports* 8 (1):11018. doi:10.1038/s41598-018-29360-3
39. Rophina M, Sharma D, Poojary M, Scaria V (2020) Circad: a comprehensive manually curated resource of circular RNA associated with diseases. *Database* 2020. doi:10.1093/database/baaa019
40. Zhao Z, Wang K, Wu F, Wang W, Zhang K, Hu H, Liu Y, Jiang T (2018) circRNA disease: a manually curated database of experimentally supported circRNA-disease associations. *Cell Death & Disease* 9 (5):475. doi:10.1038/s41419-018-0503-3
41. Xia S, Feng J, Chen K, Ma Y, Gong J, Cai F, Jin Y, Gao Y, Xia L, Chang H, Wei L, Han L, He C (2018) CSCD: a database for cancer-specific circular RNAs. *Nucleic Acids Res* 46 (D1):D925-D929. doi:10.1093/nar/gkx863
42. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene Ontology: tool for the unification of biology. *Nature Genetics* 25 (1):25-29. doi:10.1038/75556
43. The Gene Ontology C (2019) The Gene Ontology Resource: 20 years and still GOing strong. *Nucleic Acids Res* 47 (D1):D330-D338. doi:10.1093/nar/gky1055
44. Mi H, Thomas P (2009) PANTHER pathway: an ontology-based pathway database coupled with data analysis tools. *Methods Mol Biol* 563:123-140. doi:10.1007/978-1-60761-175-2_7

45. Chen H, Boutros PC (2011) VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics* 12:35. doi:10.1186/1471-2105-12-35
46. Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York,
47. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, Jin Y, Yang Y, Chen LL, Wang Y, Wong CC, Xiao X, Wang Z (2017) Extensive translation of circular RNAs driven by N(6)-methyladenosine. *Cell Res* 27 (5):626-641. doi:10.1038/cr.2017.31
48. Glazar P, Papavasileiou P, Rajewsky N (2014) circBase: a database for circular RNAs. *RNA* 20 (11):1666-1670. doi:10.1261/rna.043687.113
49. Mahmoudi E, Fitzsimmons C, Geaghan MP, Shannon Weickert C, Atkins JR, Wang X, Cairns MJ (2019) Circular RNA biogenesis is decreased in postmortem cortical gray matter in schizophrenia and may alter the bioavailability of associated miRNA. *Neuropsychopharmacology* 44 (6):1043-1054. doi:10.1038/s41386-019-0348-1
50. Ji P, Wu W, Chen S, Zheng Y, Zhou L, Zhang J, Cheng H, Yan J, Zhang S, Yang P, Zhao F (2019) Expanded Expression Landscape and Prioritization of Circular RNAs in Mammals. *Cell Rep* 26 (12):3444-3460 e3445. doi:10.1016/j.celrep.2019.02.078
51. Rybak-Wolf A, Stottmeister C, Glazar P, Jens M, Pino N, Giusti S, Hanan M, Behm M, Bartok O, Ashwal-Fluss R, Herzog M, Schreyer L, Papavasileiou P, Ivanov A, Ohman M, Refojo D, Kadener S, Rajewsky N (2015) Circular RNAs in the Mammalian Brain Are Highly Abundant, Conserved, and Dynamically Expressed. *Mol Cell* 58 (5):870-885. doi:10.1016/j.molcel.2015.03.027
52. Jeck WR, Sharpless NE (2014) Detecting and characterizing circular RNAs. *Nature Biotechnology* 32 (5):453-461. doi:10.1038/nbt.2890
53. Bahn JH, Zhang Q, Li F, Chan TM, Lin X, Kim Y, Wong DT, Xiao X (2015) The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. *Clin Chem* 61 (1):221-230. doi:10.1373/clinchem.2014.230433
54. Li H, Li K, Lai W, Li X, Wang H, Yang J, Chu S, Wang H, Kang C, Qiu Y (2018) Comprehensive circular RNA profiles in plasma reveals that circular RNAs can be used as novel biomarkers for systemic lupus erythematosus. *Clin Chim Acta* 480:17-25. doi:10.1016/j.cca.2018.01.026
55. Huang ZK, Yao FY, Xu JQ, Deng Z, Su RG, Peng YP, Luo Q, Li JM (2018) Microarray Expression Profile of Circular RNAs in Peripheral Blood Mononuclear Cells from Active Tuberculosis Patients. *Cellular Physiology and Biochemistry* 45 (3):1230-1240. doi:10.1159/000487454
56. Lasda E, Parker R (2016) Circular RNAs Co-Precipitate with Extracellular Vesicles: A Possible Mechanism for circRNA Clearance. *PLoS One* 11 (2):e0148407. doi:10.1371/journal.pone.0148407
57. Li J, Li Z, Jiang P, Peng M, Zhang X, Chen K, Liu H, Bi H, Liu X, Li X (2018) Circular RNA IARS (circ-IARS) secreted by pancreatic cancer cells and located within exosomes regulates endothelial monolayer permeability to promote tumor metastasis. *Journal of Experimental & Clinical Cancer Research* 37 (1):177. doi:10.1186/s13046-018-0822-3
58. Pan RY, Liu P, Zhou HT, Sun WX, Song J, Shu J, Cui GJ, Yang ZJ, Jia EZ (2017) Circular RNAs promote TRPM3 expression by inhibiting hsa-miR-130a-3p in coronary artery disease patients.

59. Ripke S, Neale BM, Corvin A, Walters JTR, Farh K-H, Holmans PA, Lee P, Bulik-Sullivan B, Collier DA, Huang H, Pers TH, Agartz I, Agerbo E, Albus M, Alexander M, Amin F, Bacanu SA, Begemann M, Belliveau Jr RA, Bene J, Bergen SE, Bevilacqua E, Bigdeli TB, Black DW, Bruggeman R, Buccola NG, Buckner RL, Byerley W, Cahn W, Cai G, Campion D, Cantor RM, Carr VJ, Carrera N, Catts SV, Chambert KD, Chan RCK, Chen RYL, Chen EYH, Cheng W, Cheung EFC, Ann Chong S, Robert Cloninger C, Cohen D, Cohen N, Cormican P, Craddock N, Crowley JJ, Curtis D, Davidson M, Davis KL, Degenhardt F, Del Favero J, Demontis D, Dikeos D, Dinan T, Djurovic S, Donohoe G, Drapeau E, Duan J, Dudbridge F, Durmishi N, Eichhammer P, Eriksson J, Escott-Price V, Essioux L, Fanous AH, Farrell MS, Frank J, Franke L, Freedman R, Freimer NB, Friedl M, Friedman JI, Fromer M, Genovese G, Georgieva L, Giegling I, Giusti-Rodríguez P, Godard S, Goldstein JI, Golimbet V, Gopal S, Gratten J, de Haan L, Hammer C, Hamshere ML, Hansen M, Hansen T, Haroutunian V, Hartmann AM, Henskens FA, Herms S, Hirschhorn JN, Hoffmann P, Hofman A, Hollegaard MV, Hougaard DM, Ikeda M, Joa I, Julià A, Kahn RS, Kalaydjieva L, Karachanak-Yankova S, Karjalainen J, Kavanagh D, Keller MC, Kennedy JL, Khrunin A, Kim Y, Klovins J, Knowles JA, Konte B, Kucinskas V, Ausrele Kucinskiene Z, Kuzelova-Ptackova H, Kähler AK, Laurent C, Lee Chee Keong J, Hong Lee S, Legge SE, Lerer B, Li M, Li T, Liang K-Y, Lieberman J, Limborska S, Loughland CM, Lubinski J, Lönngqvist J, Macek Jr M, Magnusson PKE, Maher BS, Maier W, Mallet J, Marsal S, Mattheisen M, Mattingdsdal M, McCarley RW, McDonald C, McIntosh AM, Meier S, Meijer CJ, Melegh B, Melle I, Meshulam-Gately RI, Metspalu A, Michie PT, Milani L, Milanova V, Mokrab Y, Morris DW, Mors O, Murphy KC, Murray RM, Myin-Germeys I, Müller-Myhsok B, Nelis M, Nenadic I, Nertney DA, Nestadt G, Nicodemus KK, Nikitina-Zake L, Nisenbaum L, Nordin A, O'Callaghan E, O'Dushlaine C, O'Neill FA, Oh S-Y, Olincy A, Olsen L, Van Os J, Pantelis C, Papadimitriou GN, Papiol S, Parkhomenko E, Pato MT, Paunio T, Pejovic-Milovancevic M, Perkins DO, Pietiläinen O, Pimm J, Pocklington AJ, Powell J, Price A, Pulver AE, Purcell SM, Quedsted D, Rasmussen HB, Reichenberg A, Reimers MA, Richards AL, Roffman JL, Roussos P, Ruderfer DM, Salomaa V, Sanders AR, Schall U, Schubert CR, Schulze TG, Schwab SG, Scolnick EM, Scott RJ, Seidman LJ, Shi J, Sigurdsson E, Silagadze T, Silverman JM, Sim K, Slominsky P, Smoller JW, So H-C, Spencer CA, Stahl EA, Stefansson H, Steinberg S, Stogmann E, Straub RE, Strengman E, Strohmaier J, Scott Stroup T, Subramaniam M, Suvisaari J, Svrakic DM, Szatkiewicz JP, Söderman E, Thirumalai S, Toncheva D, Tosato S, Veijola J, Waddington J, Walsh D, Wang D, Wang Q, Webb BT, Weiser M, Wildenauer DB, Williams NM, Williams S, Witt SH, Wolen AR, Wong EHM, Wormley BK, Simon Xi H, Zai CC, Zheng X, Zimprich F, Wray NR, Stefansson K, Visscher PM, Trust Case-Control Consortium W, Adolfsson R, Andreassen OA, Blackwood DHR, Bramon E, Buxbaum JD, Børglum AD, Cichon S, Darvasi A, Domenici E, Ehrenreich H, Esko T, Gejman PV, Gill M, Gurling H, Hultman CM, Iwata N, Jablensky AV, Jönsson EG, Kendler KS, Kirov G, Knight J, Lencz T, Levinson DF, Li QS, Liu J, Malhotra AK, McCarroll SA, McQuillin A, Moran JL, Mortensen PB, Mowry BJ, Nöthen MM, Ophoff RA, Owen MJ, Palotie A, Pato CN, Petryshen TL, Posthuma D, Rietschel M, Riley BP, Rujescu D, Sham PC, Sklar P, St Clair D, Weinberger DR, Wendland JR, Werge T, Schizophrenia Working Group of the Psychiatric Genomics C, Psychosis Endophenotypes International C (2014) Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511 (7510):421-427. doi:10.1038/nature13595

60. Shao Y, Li J, Lu R, Li T, Yang Y, Xiao B, Guo J (2017) Global circular RNA expression profile of human gastric cancer and its clinical significance. *Cancer Med* 6 (6):1173-1180. doi:10.1002/cam4.1055
61. Yao G, Niu W, Zhu X, He M, Kong L, Chen S, Zhang L, Cheng Z (2019) hsa_circRNA_104597: a novel potential diagnostic and therapeutic biomarker for schizophrenia. *Biomark Med* 13 (5):331-340. doi:10.2217/bmm-2018-0447
62. Maffioletti E, Cattaneo A, Rosso G, Maina G, Maj C, Gennarelli M, Tardito D, Bocchio-Chiavetto L (2016) Peripheral whole blood microRNA alterations in major depression and bipolar disorder. *J Affect Disord* 200:250-258. doi:10.1016/j.jad.2016.04.021
63. van den Berg MMJ, Krauskopf J, Ramaekers JG, Kleinjans JCS, Prickaerts J, Briede JJ (2020) Circulating microRNAs as potential biomarkers for psychiatric and neurodegenerative disorders. *Prog Neurobiol* 185:101732. doi:10.1016/j.pneurobio.2019.101732
64. He K, Guo C, He L, Shi Y (2018) MiRNAs of peripheral blood as the biomarker of schizophrenia. *Hereditas* 155:9. doi:10.1186/s41065-017-0044-2
65. Gardiner E, Beveridge NJ, Wu JQ, Carr V, Scott RJ, Tooney PA, Cairns MJ (2012) Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells. *Mol Psychiatry* 17 (8):827-840. doi:10.1038/mp.2011.78
66. Zimmerman AJ, Hafez AK, Amoah SK, Rodriguez BA, Dell'Orco M, Lozano E, Hartley BJ, Alural B, Lalonde J, Chander P, Webster MJ, Perlis RH, Brennand KJ, Haggarty SJ, Weick J, Perrone-Bizzozero N, Brigman JL, Mellios N (2020) A psychiatric disease-related circular RNA controls synaptic gene expression and cognition. *Molecular Psychiatry* 25 (11):2712-2727. doi:10.1038/s41380-020-0653-4
67. Han D, Li J, Wang H, Su X, Hou J, Gu Y, Qian C, Lin Y, Liu X, Huang M, Li N, Zhou W, Yu Y, Cao X (2017) Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression. *Hepatology* 66 (4):1151-1164. doi:10.1002/hep.29270
68. Li K, Wan CL, Guo Y (2020) Circular RNA circMTO1 Suppresses RCC Cancer Cell Progression via miR9/LMX1A Axis. *Technol Cancer Res Treat* 19:1533033820914286. doi:10.1177/1533033820914286
69. Zhang X, Zhong B, Zhang W, Wu J, Wang Y (2019) Circular RNA CircMTO1 Inhibits Proliferation of Glioblastoma Cells via miR-92/WWOX Signaling Pathway. *Med Sci Monit* 25:6454-6461. doi:10.12659/MSM.918676

Figures

Figure 1

Analysis of differential expression of circRNA. (A) Changes in circRNA expression profile in SZ samples (left) and BD samples (right) as compared to HC individuals. (B) Alteration of CLR in SZ as compared to HC samples; the red dots denote significant change and black dots non-significant change. The red dots indicate significant upregulation and green dots represent downregulation, with the vertical lines corresponding to 2-fold (A) and 1.5-fold (B) change and the horizontal line representing a cut-off Pvalue <0.0036.

Figure 2

Validation of differentially expressed circRNA by qPCR. (A) The relative expression of specific circRNAs in PBMC samples of schizophrenia (SZ) and bipolar disorder (BD) (B) compared to healthy controls (HC). There were 21 samples in each group. Data are shown as median and IQR for three independent experiments. CircRNAs expression was normalized using GAPDH and HMBS as control genes. Statistical significance was performed by Student's t-test (one-tailed). *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 3

Alternative splicing analysis of circRNA. (A) The overlap of the AS events in SZ, BD and HC groups. (B) Composition of AS types, including exon skipping (ES), intron retention (IR), and alternative 5 or 3 splicing site (A5SS and A3SS), in each diagnosis group. (C) Heatmap of relative abundance of the AS events (PSI) for all the samples showed three distinct groups with the top group (purple bar) including 26 events were broadly expressed across the diagnosis groups.

Figure 4

Characterization of circRNA in human PBMC. (A) Validation of the detected circRNAs (n=15) using qPCR. Data are shown as mean $\pm$ SEM and all reactions were in triplicates in which GAPDH gene was used as reference. (B) Pathway overrepresentation analysis for the parental genes of the identified circRNAs. (C) Heatmap of average CLR in across the studied groups.

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