Published in final edited form as:

Prog Neuropsychopharmacol Biol Psychiatry. 2017 February 6; 73: 109–118. doi:10.1016/j.pnpbp.2016.02.009.

The Schizophrenia Risk Gene MIR137 Acts as a Hippocampal Gene Network Node Orchestrating the Expression of Genes Relevant to Nervous System Development and Function

Nikkie FM Olde Loohuis^{1,2}, Nael Nadif Kasri^{1,2,3}, Jeffrey C Glennon^{1,2}, Hans van Bokhoven^{1,2,3}, Sébastien S Hébert^{4,5}, Barry B. Kaplan⁶, Gerard JM Martens^{2,7}, and Armaz Aschrafi^{2,6}

¹ Department of Cognitive Neuroscience, Radboudumc, 6500 HB Nijmegen, the Netherlands ² Donders Institute for Brain, Cognition, and Behaviour, Centre for Neuroscience, 6525 AJ Nijmegen, the Netherlands ³ Department of Human Genetics, Radboudumc, 6500 HB Nijmegen, the Netherlands ⁴ Axe Neurosciences, Centre de recherche du CHU de Québec, CHUL, Québec, QC, G1V4G2, Canada ⁵ Département de psychiatrie et neurosciences, Université Laval, Québec, QC, G1V 0A6, Canada ⁶ Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland, 20892, USA ⁷ Department of Molecular Animal Physiology, Radboud University Nijmegen, 6525 HP Nijmegen, the Netherlands

Abstract

MicroRNAs (miRs) are small regulatory molecules, which orchestrate neuronal development and plasticity through modulation of complex gene networks. microRNA-137 (miR-137) is a brain-enriched RNA with a critical role in regulating brain development and in mediating synaptic plasticity. Importantly, mutations in this miR are associated with the pathoetiology of schizophrenia (SZ), and there is a widespread assumption that disruptions in miR-137 expression lead to aberrant expression of gene regulatory networks associated with SZ. To systematically identify the mRNA targets for this miR, we performed miR-137 gain- and loss-of-function experiments in primary rat hippocampal neurons and profiled differentially expressed mRNAs through next-generation sequencing. We identified 500 genes that were bidirectionally activated or repressed in their expression by the modulation of miR-137 levels. Gene ontology analysis using two independent software resources suggested functions for these miR-137-regulated genes in neurodevelopmental processes, neuronal maturation processes and cell maintenance, all of which known to be critical for proper brain circuitry formation. Since many of the putative miR-137 targets identified here also have been previously shown to be associated with SZ, we propose that this miR acts as a critical gene network hub contributing to the pathophysiology of this neurodevelopmental disorder.

***Corresponding author:** Dr. Armaz Aschrafi, Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland, 20892, USA; armaz.aschrafi@nih.gov; Phone Number: +1-301-451-4251.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

gene network; microRNA; neurodevelopment

1. Introduction

Numerous recent studies have suggested that critical events during synaptic development and function are dependent on the activity of a large number of proteins, and that disturbance of individual components within a protein network or alterations of their activities causes synaptic dysfunction, phenotypically culminating in schizophrenia (SZ) and other neurological disorders.¹⁻³ Thus far, regulation at the level of transcription and translation has been assumed to be important for proper synaptic function. Recent exploration of genes that impair synaptic function and increase the risk for developing SZ has led to the investigation of small non-coding (nc) microRNAs (miRs).⁴ This is based on numerous observations, for example, a subset of brain-enriched miR genes located within an imprinted cluster on chromosome 14q32 is differentially expressed in a large cohort of SZ patients;⁵ SZ pathophysiology is partially based on the pattern of miR dysregulation emerging due to 22q11.2 deletions, which is shaped by the combined effect of miR-185 and Dgcr8 hemizyosity.⁶ Recently, common genetic variants and rare genomic deletions associated with miR-137 have been associated with severe cognitive impairments in the context of SZ and intellectual disability (ID).⁷⁻¹⁰ GWAS studies have strongly implicated the single nucleotide polymorphism (SNP) rs1625579 located in an intron of *MIR137* in the aetiology of SZ.^{10, 11} Homozygosity for this SNP was significantly associated with lower miR-137 expression in postmortem prefrontal cortex tissue.⁷ Interestingly, risk alleles on the *MIR137* SNPs predicted SZ phenotypic variabilities, such as age-at-onset of psychosis and brain structure, and negative symptoms of cognitive subtypes of SZ.¹²⁻¹⁵ In addition, SZ-associated SNPs have been found in predicted targets of miR-137, such as *CSMD1*, *C10orf26*, *CACNA1C* and *TCF4*.⁹ In induced neurons derived from fibroblast of SZ patients, miR-137 expression is found increased and its target genes, including *CPLX1*, *NSF*, *SYN3* and *SYT1*, were expressed at lower levels compared to cells derived from healthy controls. As a functional consequence, synaptic vesicle release was disturbed in these cells, a phenotype which was confirmed using *in vivo* studies in mice with manipulated miR-137 expression levels in the hippocampus.¹⁶ Moreover, we recently identified ID patients with chromosome 1p21.3 microdeletions comprising *MIR137*, resulting in haploinsufficiency of miR-137.¹⁷

miR-137 is a synaptically enriched miR,¹⁷ that acts as a negative regulator of dendrite morphogenesis and spine development in newborn neurons.¹⁸ In mature neurons, miR-137 has been shown to play a role in presynaptic vesicle release at the DG-CA3 synapse, by targeting complexin-1 (*Cplx1*), N-ethylmaleimide-sensitive fusion protein (*Nsf*) and synaptotagmin-1 (*Syt1*).¹⁶ In addition, a critical role was revealed for miR-137 in controlling synaptic strength at the CA3-CA1 synapse and genetic defects in *MIR137* were linked to glutamatergic dysfunction, which may ultimately contribute to the pathogenesis of SZ-associated cognitive impairments.¹⁹

Since miRs can target up to hundreds of different mRNAs, there is a need to delineate the full spectrum of genes regulated by miR-137 in order to understand how these multiple targets interact together, and to what extent each of them contributes to assigned miR-137's function in neuronal development or dysfunction.^{20, 21} Previously, Wright et al. used a combination of literature searches and various bioinformatics resources to analyze the possible role for miR-137 in SZ.²² This *in silico* analysis revealed an overrepresentation of miR-137 targets in different facets of nervous system development and function, including synaptic long-term potentiation, ephrin receptor signaling and axonal guidance.

In the present study we tested the hypothesis that pathways regulated by miR-137 are important in the aetiology of SZ. For the evaluation of this hypothesis we set out experiments to identify the biological targets of miR-137, which are largely unknown. Toward this end, we have combined computational approaches with whole-genome mRNA expression profiling in cultured hippocampal neurons in which miR-137 was either up- or downregulated. Collectively, our studies point toward miR-137 as a critical modulator of pathways involved in neuronal development and cognitive function, by acting as a post-transcriptional regulator of genes involved in neuro-developmental and neuronal maturation processes as well as cell maintenance, at least in part, through trans-membrane receptor signaling and regulation of the actin cytoskeleton.

2. Materials and Methods

2.1. Animals

Wistar rats (Harlan laboratories, USA) were housed in groups of 2 or 3 animals on a 12-h light cycle in a temperature-controlled ($21 \pm 1^\circ\text{C}$) environment with ad libitum access to food and water. Rats were used at embryonic day E18 for the preparation of primary neuronal cultures. All experiments involving animals were evaluated and approved by the Committee for Animal Experiments of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

2.2. Primary neuronal cultures

All media and reagents were purchased from Life Technologies. Dissociated hippocampal cultures were prepared from rats at embryonic day E18. Pregnant rats were anaesthetized with isoflurane and sacrificed by cervical dislocation. Embryonic brains were dissected in ice-cold Hank's buffer (HBSS without Ca^{2+} and Mg^{2+} , 100 U/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, $1 \times$ GlutaMax). From 5-6 rat pups, the hippocampi were separated from the cortices and, pooled together and incubated with 0.025% trypsin for 15 min at 37°C . Neurons were mechanically dissociated in NeuroBasal medium containing 10% FCS and $1 \times$ GlutaMax (Invitrogen) using fire-polished Pasteur pipettes. The cell suspension was filtered through a $70 \mu\text{m}$ cell strainer (BD Falcon) and plated on poly-D-lysine (Invitrogen, 70.000-150.000) pre-coated glass coverslips in 24-wells plates at a density of 25.000 neurons per well. Medium was replaced after 6 hours with Neurobasal medium containing B27 supplement, and $1 \times$ GlutaMax. Neurons were maintained in a 5% CO_2 humidified 37°C incubator. One-third of the medium was refreshed every three to four days.

2.3. miR constructs and validation

For a detailed description of the sponge-miR-137 and oe-miR-137 constructs design and validation we refer to Olde Loohuis et al.¹⁹

2.4. Virus production

Viral particles were prepared by co-transfection of HEK-293T cells with a FUGW plasmid, the HIV-1 packaging vector 8.9, and the VSVG envelope glycoprotein vector using the calcium phosphate co-precipitation method. HEK293T cells were placed overnight in a 3% CO₂ humidified 37°C incubator. The following day, medium was replaced by fresh ultraculture (Lonza) containing 4 mM valproate (Sigma) and the CO₂ percentage was set to 10 %. Supernatants were collected 24 h and 48 h later. Viral particles were purified over a 20 % sucrose cushion by ultracentrifugation for 3 h at 21.000 RPM. Viral particles were resuspended in PBS without Ca²⁺ and Mg²⁺ (Life technologies), and stored at -80°C until use.

2.5. RNA Sequencing

Primary hippocampal neurons were infected with either control, sponge-miR-137 or overexpressing miR-137 (oe-miR-137) lentiviruses at 8 DIV. Neurons were derived from the same nest of rat pups for all conditions. At 21 DIV total RNA was extracted from three independent cultures per condition using the miRNeasy Mini Kit following manufacturer instructions (Qiagen). RNA quality was assessed using TapeStation analysis, which revealed RNA quality above RIN 9.0 for all samples. For each condition, equal fractions of RNA from these three cultures were pooled into 1 sample, up to a total of 500 ng RNA per condition. The individual samples were kept for qPCR validation. The pooled samples for RNA sequencing purposes were further processed by the HudsonAlpha Institute for Biotechnology (Huntsville, AL, USA). The RNA sequencing was performed in three separate flow cells generating a total of 45 to 50 million 50 bp reads per sample. RNA sequencing data analysis was carried out with the GeneSifter gene expression analysis suite (Geospiza, Seattle, WA, USA). Expression data was normalized against the total number of mapped reads. The reads were assembled into genes. Differential gene expression between pooled samples of neurons infected with the control virus and sponge-miR-137 or miR-137 was determined using the likelihood ratio test and the Benjamini and Hochberg correction for multiple testing was applied. Thresholds were set at an Reads Per Kilobase of Transcript Per Million Reads Mapped (RPKM) of 1.0 and a fold change of 1.2. Genes were assigned differentially expressed at an adjusted P-value $P < 0.05$.

2.6. Gene Ontology (GO) analysis

Bidirectionally affected genes in sponge-miR-137 and miR-137 infected neurons compared to control neurons were overlaid onto ontological pathways using both DAVID software²³ and Ingenuity Pathway Analysis® to provide insights into biological pathways affected by miR-137 manipulation. We performed a two-step analysis consisting of (1) DAVID KEGG pathway analysis and IPA canonical pathway analysis to identify pathways in which miR-137 regulated genes are involved, and (2) DAVID GO-term based clustering and IPA

Function and Disease analysis to identify cellular processes that are affected by miR-137. Outcomes from both analyses were overlaid to come to a consensus.

2.7. Quantitative Real-Time PCR (RT-qPCR) Analysis

Total RNA was extracted from primary neurons using the miRNeasy Mini Kit following manufacturer instructions (Qiagen). Total RNA was reversed-transcribed using the revertAid™ H Minus First Strand cDNA Synthesis kit (Fermentas Inc., USA) according to manufacturer's protocol qPCR was performed using bioline SYBR Green (Bioline). mRNA expression was normalized to rat U6 and β -actin using the GeNorm method.²⁴ Each sample was run at least three independent times. Primer sequences are presented in the supplementary information.

2.8. Statistical analysis

Statistical analyses were performed using Prism software (Graphpad). Statistical tests were used with alpha set at the 95% confidence interval, i.e. $p < 0.05$. Two group comparisons were evaluated using a one-way ANOVA followed by Tukey's post-hoc analysis. Data are plotted as mean $\pm$ SEM. Quantification was performed in a blinded fashion.

3. Results

3.1. Transcriptome analysis of miR-137-manipulated primary hippocampal neurons

In order to investigate gene expression networks controlled by miR-137, we selectively reduced or increased miR-137 levels in primary rat hippocampal neurons. To generate a miR-137 loss-of-function condition, a previously validated lentiviral-based eGFP-vector was modified to express miR-137 sequestering sites (sponge-miR-137), whereas the gain-of-function construct (oe-miR-137) contains the mouse precursor miR-137 sequence (suppl. figure 1A). Infection of primary hippocampal neurons with sponge-miR-137 and oe-miR-137 lentiviruses led to a nearly 100% infection efficiency (suppl. figure 1B).

Consistent with previous findings for other small RNAs,²⁵ RNA sequencing revealed that modulating miR-137 levels in neurons resulted in alterations in the expression of hundreds of mRNAs, as compared to mock-infected neurons. The distributions of gene expression in loss- and gain-of-function studies in hippocampal neurons is shown in figure 1 A and B. Suppression of miR-137 resulted in an asymmetrical distribution of genes towards an upregulation of mRNA expression levels (figure 1A). Overexpression of miR-137 had the opposite effect, thus downregulating the expression of most genes (figure 1B). For the differential expression analysis, the adjusted P-value threshold was set at 0.05. While suppression of miR-137 resulted in the significant alteration of 3066 transcripts in total (1846 genes upregulated, 1220 genes downregulated), miR-137 overexpression changed the levels of 2797 transcripts (1084 genes upregulated, 1713 genes downregulated).

With an expression-level threshold of RPKM ≥ 1.0 in at least one condition, miR-137 overexpression resulted in the decreased expression of 1263 genes and elevated the expression levels of 305 genes (adjusted P-value < 0.05 and fold change ≥ 1.2 in at least one condition). Conversely, sponge-mediated miR-137 suppression enhanced the levels of 1212

transcripts, while decreasing the levels of 400 mRNAs. Consistent with previous findings that miR overexpression results in enhanced turn-over of putative target mRNAs, whereas miR suppression elevates the levels of downstream target mRNAs,²⁶ our data revealed that among the 1.2-fold changed genes (for either the up- or downregulated set of genes), the levels of 500 genes were both upregulated in sponge-miR-137 neurons and downregulated in miR-137 overexpressing neurons (suppl. table 1A). In the sponge-miR-137 condition 712 genes are uniquely upregulated, while 763 genes are downregulated in the oe-miR-137 neurons (figure 1C). In addition, 373 unique genes showed decreased expression levels in sponged neurons and 278 genes were upregulated in the overexpression condition, while 27 genes showed increased expression levels in the overexpression condition and decreased expression in the sponge-miR-137 condition (figure 1C). To define the miR-137-regulated transcripts, we compared the bidirectionally regulated transcripts to the sets of putative miR-137 targets computationally predicted by the miRanda,²⁷ miRDB,^{28, 29} miRWalk³⁰ and TargetScan.³¹ A total of 40 (or 8%) out of the 500 bidirectionally expressed transcripts contained at least one predicted binding site, while only two of the 27 inversely regulated transcripts (7.4%) contained a miR-137 seed-matching sequence in their respective 3'UTRs (figure 1C; suppl. table 1B). This low percentage of transcripts with a miR-137 binding site suggested that miR-137 may bind to these bidirectionally regulated miRs in a non-canonical manner, as was recently demonstrated for other miRs;³² it is also likely that a significant proportion of the altered transcripts were modulated by miR-137 via a secondary downstream effect.

3.2. Validation of RNA sequencing results by RT-qPCR

We used RT-qPCR analysis to validate the outcome of the RNA sequencing, and chose eight genes that had a wide range of expression (from RPKMs between 0.24 and 49.3, and fold changes between 1.19 and -4.83). These genes were upregulated in the sponge-miR-137 condition and concomitantly downregulated due to miR-137 overexpression. The selected genes (*Arpc1b*, *Ppp1r14b*, *Cspg4*, *Tjp2*, *Cdc42ep1*, *F2r*, *Fgfr3* and *Pdgfra*) encode receptors, intracellular signaling molecules and actin-binding proteins. As in the RNA sequencing, we maintained a fold change of 1.2 as the threshold for differential gene expression.

For the sponge-miR-137 condition, miR-137-mediated upregulation of five out of eight genes was confirmed using RT-qPCR (*Arpc1b* $P < 0.01$; *Cspg4* $P < 0.05$; *F2r* $P < 0.01$; *Fgfr3* $P < 0.01$; *Ppp1r14b* $P < 0.05$), whereas two genes showed a tendency towards increased expression (*Tjp2*; *Pdgfra*), and the expression of one gene was not increased (*Cdc42ep1*) (figure 1D; suppl. table 1C). For the oe-miR-137 condition, the levels of five out of the same eight genes could be validated by RT-qPCR (*Arpc1b* $P < 0.01$; *F2r* $P < 0.05$; *Fgfr3* $P < 0.01$; *Ppp1r14b* $P < 0.01$; *Cdc42ep1* $P < 0.01$), whereas the expression of the other three genes (*Cspg4*; *Tjp2*; *Pdgfra*) exhibited a tendency towards downregulation (figure 1D; suppl. table 1C). Overall, the RNA sequencing data and the outcome of the qPCR analysis pointed toward similar alteration levels with regard to differential miR-137 induced gene expression.

3.3. Identification of miR-137 targets genes

In order to identify the main signaling pathways and cellular processes in which the 500 miR-137 affected genes are involved, we used two different gene ontology (GO) analyses,

DAVID and Ingenuity Pathway Analysis (IPA). As a first step, canonical pathway analysis by IPA and KEGG pathway analysis by DAVID were performed to identify the most relevant functional pathways that are enriched in the 500 bidirectionally miR-137 modulated genes. DAVID-based analysis revealed a significant enrichment for 'focal adhesion' and 'extracellular matrix (ECM)-receptor interaction' pathways, whereas IPA pointed towards enrichment for pathways in 'Human embryonic stem cell pluripotency', 'Integrin Linked Kinase (ILK) signaling' and 'Hepatic Fibrosis / Hepatic Stellate Cell Activation' (suppl. table 2A, B). Although the pathways identified using these two software's are clearly different, 50% of the genes within the top pathways by DAVID and IPA were identical (additional information can be found in the suppl. text 'detailed description of the molecular network'). Suppl. figure 2 shows that genes in the top processes mainly represent membrane bound proteins relative to the number of extracellular and intracellular proteins being affected by miR-137. Secondly, the investigation revealed that ECM-receptor interaction, focal adhesion, regulation of the actin cytoskeleton and subsequently MAPK pathways could be major signaling pathways affected by miR-137.

To examine the cellular processes in which the miR-137 affected genes are involved, additional analyses were performed using the 'Function and Disease' analysis in IPA and the GO-term based clustering analysis in DAVID. This analysis aimed at identifying the biological processes (BP), cellular component (CC) and molecular function (MF). This approach resulted in three top processes in both IPA (cardiovascular system development and function; organismal development and cellular movement) and DAVID (response to wounding; extracellular region part; cytoskeletal protein binding) (suppl. table 2C). The genes involved in these pathways are shown in suppl. table 2D. Genes associated with organismal development were completely overlapping with the genes involved in cardiovascular system development and function. Subsequent literature research was performed to identify the role of the 126 genes represented in the IPA top processes, thereby distinguishing between neuronal and glial cells (suppl. table 3A-D). In addition, the genes derived from the IPA and DAVID pathway analyses were included in this study (marked with an asterix). Four main cellular themes were found enriched, being (1) Neurodevelopmental processes, including proliferation, migration and differentiation (figure 2), (2) Neuronal maturation processes, including neurite extension, axon outgrowth, dendritic tree morphology and spine and synapse functioning (figure 3), (3) Cellular maintenance, including survival, apoptosis and protection (figure 4), (4) Intracellular signaling, including MAPK, ERK, RhoA, Pi3K and STAT pathways (figure 5). Detailed gene-specific information used to draw the functional theme figures is provided in suppl. table 3A-D.

Previous studies have strongly suggested that aberrant miR-137 expression may result in changes in the expression of target genes associated with neurological disorders, such as in SZ.^{8,9} Hence, we compared the list of the 500 miR-137 bidirectionally modulated genes with a list of reference genes affected in SZ which was derived from SZGR database (bioinfo.mc.vanderbilt.edu/SZGR), and consisted of a total of 694 genes (suppl. table 4A). We found that 31 genes identified are modulated by miR-137 as present in the SZ gene list (suppl. table 4B), 12 of which (Add3, Cald1, Cdc42ep1, Ddr1, Ezr, Fermt2, Itga7, Myo1b,

Pdlim5, S1pr1, Slc1a2 and Sspn) can participate in the ECM-receptor interaction pathway or act on the actin cytoskeleton.

4. Discussion

Since alteration in genes regulated by miR-137 coupled with dysregulation by this miR may synergistically contribute to SZ risk, we set out to examine whether miR-137 has the capacity to act as a regulatory gene network node in hippocampal neurons. This investigation revealed a large number of genes involved in neurodevelopmental pathways, including the regulation of actin cytoskeleton and ECM receptor signaling, and cellular processes including neuronal maturation and cell maintenance. These findings raise the possibility that these pathways and processes in concert contribute to impaired synaptic development and function.

In this study we verified the involvement of miR-137 affected genes in multiple neurodevelopmental processes, including cell migration, proliferation and differentiation. These processes are partially controlled by ERK and MAPK signaling pathways, which were also found enriched in our dataset of bidirectionally regulated genes. Lang et al. (2014) recently showed that SZ is associated with deletions of *ULK4*, leading to changes in intracellular signaling through ERK and MAPK pathways.³³ They further provide evidence for disturbances in the regulation of cell migration, and neurite extension and branching due to ULK4-induced disturbances in ERK and MAPK signaling. In addition, a recent genetics-based study showed that 16 SNPs within 10 genes of the MAPK pathway (*ATF1*, *DAXX*, *FOS*, *HRAS*, *JUN*, *MAP2K1*, *MAPK1*, *MAPK3*, *TRAF2* and *YWHAB*) were significantly associated with SZ.³⁴ Furthermore, studies assessing protein expression and mRNA expression on members of the MPK pathway,³⁵⁻⁴⁰ revealed associations between SZ and dysregulation of this pathway in several brain regions.^{36, 40} These members include *ERK1/2*,^{36, 37, 38} *JNK1* and *JNK2*,^{35, 36} *ATF-2*,³⁹ *CREB*,^{38, 39} *c-Fos* and *c-Jun* Fos B, *Jun B* and *Jun D*,^{36, 40} *MKP2*,³⁷ *B-raf*, *MEK1*, *MEK2*, *RSK1*, and *Rap1*.³⁸ Altogether, our studies provide evidence for the association of disturbances in ERK and MAPK signaling pathways with SZ, and that dysregulation of these pathways could be related to miR-137 expression.

In addition, in our dataset of miR-137 affected genes the process of apoptosis was found enriched, which includes *TGF- β* , *BMP6* and *BAG3*. Recently, in pyramidal neurons from the superior temporal gyrus of SZ patients, apoptosis-related genes were found dysregulated compared to healthy controls and these genes included BMPs, *TGF- β* and members of the *Bcl-2* pathway⁴¹, thereby suggesting that miR-137 may be involved in the neuronal apoptosis-related aspect of this neurodevelopmental disease.

Previous research already suggested that defects in the ECM receptor interaction and actin cytoskeleton pathways may underlie disturbances in proper memory formation,⁴² and may be causative for ID and SZ.^{43, 44} In our study, the expression of multiple integrins was altered suggesting a role for miR-137 in the modulation of integrin signaling within the ECM pathway. In neurons, integrin signaling leads to cytoskeletal changes resulting in altered growth cone modulation, neurite outgrowth and axon migration,⁴⁵⁻⁴⁷ as well as regulation of calcium signaling,⁴⁸⁻⁵⁰ activation of glutamate receptors,⁵¹ gene transcription,

and the release of neurotransmitters.⁴³ Indeed, neurite outgrowth and axon extension are GO terms being enriched in our dataset, whereas miR-137 was previously experimentally linked to proper AMPAR signaling and presynaptic vesicle release.^{16,19} Differential expression of multiple integrins due to the modulation of a given miR may thus result in major effects on neuronal functioning culminating in neurological disorders. Indeed, olfactory neurosphere-derived cells from SZ patients exhibited increased integrin-dependent cell motility, while application of integrin antibodies against ITGA8 and ITGB1, which dimerize to interact with fibronectin (FN), reduced cell motility of the patient-derived cells to basal levels.⁵² In addition, disturbances in gene expression levels from the ECM signaling pathway were identified in post-mortem brain tissue of SZ patients, including expression changes in CSPGs, MMPs and integrins. Notably, the expression levels of several integrins, including Itgb1, CSPGs and FN, were bidirectionally regulated by miR-137 as revealed in our study. Other ECM-associated genes affected in SZ patient-derived cells and that we also identified as miR-137 targets are COL6A2 and PDGFRA.

Lastly, accumulating evidence suggest dysregulation of the actin cytoskeleton in SZ,^{41, 52} including altered expression levels of the actin-cytoskeleton associated genes PARVB and DOCK1, in agreement with our finding in the miR-137 manipulated neurons.⁵² Actin-binding proteins, including Parvb and Dock1, but also Filamins, Talin and Fermt2 regulate actin polymerization, stabilization, elongation and branching.^{42, 53, 54} Reorganizations of the actin cytoskeleton are essential for memory formation since they underlie the modulation of events such as presynaptic vesicle movement, postsynaptic glutamate receptors trafficking and dendritic spines morphogenesis.⁴² Altered expression of miR-137 could as such impair neuronal and synaptic functioning, contributing to the cognitive deficits in associated integrity of neuronal networks in neuropathology.

In line with the study presented here, two recent studies have examined putative miR-137 target genes by overexpressing or inhibiting miR-137 *in vitro* and profiling the resulting mRNA expression pattern.^{55, 56} In comparison with the study of Hill et al.⁵⁶, we find a 4.4% (22 genes) overlap with our bidirectionally changed genes and their miR-137 loss-of-function condition, and a 5.2% (26 genes) overlap with their overexpression condition. For the study by Collins et al.⁵⁵, in which the effects of overexpression of miR-137 were studied, we find a 7% overlap (35 genes) with our bidirectionally changed genes, of which only 2 genes (Notch3 and Plp1) were also identified in the overexpression condition as described by Hill et al. A possible explanation for the lack in common miR-137 target genes between our studies could lie within the cellular system used to study miR-137 effects. Whereas Collins et al.⁵⁵ and Hill et al.⁵⁶ used neuronal progenitor cells, we made use of mature primary hippocampal neurons, a decision based on the synaptic phenotype of miR-137 deregulation.¹⁹ Further reasons likely to cause the discrepancies in mRNA profiles could be the time intervals between induction of miR-137 gain- or loss-of function, and the time point of cell lysis for subsequent total RNA isolation and RNA profiling.

The high-throughput mapping of the gene network interacting with miR-137 identified 500 genes that were bidirectionally repressed or derepressed by modulation of miR-137 levels in hippocampal neurons. In our study, many of the high-confidence miR-137 sites previously identified could not be assigned in our whole-transcriptome analysis, while the non-seed-

mediated interactions constituted the largest class in our data. This outcome seems surprising, since computational prediction of seed binding is commonly used to assign miR-mRNA interactions. The substantial class of miR-137 interactions that did not rely on miR-137 binding sites suggests that non-canonical miR-137 interactions have additional functions, possibly in attracting regulatory factors or switching effector pathways, as was recently suggested for other miRs.³² Indeed, it was proposed that differences in patterns of miR-target RNA base pairing may induce allosteric changes in the RISC complex, potentially leading to different interaction classes and/or the specific motifs identified might have distinct functional roles.⁵⁷ Moreover, effects of miRs on steady-state levels of target mRNAs can be complicated by feedback regulation, as previously shown for other miRs.⁵⁸ Feedback regulation of miR targets may provide a sensitive mechanism for fine-tuning the expression of target genes and, coupled with translational repression, for modulating the expression of proteins encoded by target genes.⁵⁹ Furthermore, miRs can have specific effects on the formation of protein complexes by selecting specific components of the complex. A positive correlation between protein connectivity and number of different targeting miRs was observed indicating that hub or functionally related proteins require more miR-mediated regulation.⁶⁰ On the other hand, the relatively low number of mRNAs containing high-confidence miR-137 sites that was confirmed by our study is supported by the notion that miRs can inhibit protein translation of their target mRNAs through miRISC-dependent release of translation initiation factors, rather than degrading its target mRNAs.⁶¹ For example, we previously demonstrated that miR-137 regulates GluA1 protein levels in primary hippocampal neurons thereby affecting synaptic maturation and plasticity while GluA1 mRNA levels were found unaffected by miR-137.¹⁹ Accordingly, the RNA sequencing data showed no effect of miR-137 manipulation on GluA1 mRNA expression. These indirect effects of miRs may also contribute to an explanation for the discrepancies in miR-137 targets found between our study using primary hippocampal neurons in which miR-137 levels were manipulated as compared to Wright et al.,⁶² where GWAS data from SZ patients was compared to potential miR-137 targets identified using merely prediction software.

Several studies have identified miRNAs as candidate biomarkers for diagnosis of SZ. Shi et al. (2012) suggested that miR-181b, miR-219-2-3p, miR-346, miR-195, miR-1308, miR-92a, miR-17, miR-103 and let-7g are the key players to reflect the SZ illnesses status,⁶³ while increased expression levels of miR-132, miR-195, miR-30e and miR-7 in plasma, and miR-212, miR-34a and miR-30e in peripheral blood mononuclear cells (PBMCs) was recently reported, this study concluded that miR-30e in plasma is the best potential biomarker.⁶⁴ Moreover, PBMCs of SZ patients showed increased expression miR-1273d, miR-1303, miR-21, miR-3064-5p, miR-3131, miR-3687, miR-4428, miR-4725-3p, miR-5096 suggesting that a combination of these miRNAs could serve as potential biomarkers.⁶⁵ Up to date, no evidence has been obtained for miR-137 as a diagnostic marker¹⁶. Interestingly, Yu et al. showed antipsychotic treatment effects on miRNA expression levels.⁶⁶ Clearly, the number of studies investigating miRNAs as potential biomarkers in SZ is increasing, however they should be interpreted with great care, since different studies on the same disease show limited overlap, possibly due to discrepancies in methodologies.⁶⁷

Since miRNAs have multiple targets, rescuing the expression levels of a single dysregulated miRNA could restore the misexpression of all its targets, possibly reversing the disease phenotype.⁶⁸ While no miR therapies are yet available for SZ patients, for several disorders phase I and II clinical trials using oligonucleotide-based strategies are ongoing, including the use of miRNA-based treatments for heart failure,⁶⁹ amyotrophic lateral sclerosis (ALS),⁷⁰ and hepatitis C.⁷¹ Challenges to be overcome concerning oligonucleotide-based treatments for SZ include the brain-specific or systemic delivery of the miR-based drug,⁶⁸ the optimum working range of the miRs, and the turn-over of the miR or the miR inhibitor which will determine the lasting of the effect.

The major limitation of our investigation to delineate the targets of miR-137 in hippocampal neurons is shared with many other studies that employ differential gene expression to identify miRNA targets. Our approach identified changes in transcript abundance, information we employed to describe the predominate genes and pathways affected by miR-137, but did not distinguish between direct and indirect targets. Consequently, many previous direct miR-137 targets others and we have identified, and functionally characterized, are missing in the present study. To identify direct miR-137 targets, computationally seed matches were examined to enrich for direct miR-137 targets over secondary effects, however, the identification of direct targets remains challenging because of modest effects on levels of some target mRNAs, as well as and the fact that some target inhibition may occur at the level of translational repression.⁷²

5. Conclusion

This study provides insight into the functional gene networks related to changes in miR-137 levels. We show that miR-137 acts as a gene network hub coordinating the expression of several components of pathways relevant to synaptic development and physiology. Among the subset of targets involved in nervous system development and function, the top scoring pathways were ECM-receptor interaction and the regulation of the actin cytoskeleton processes that are critical for proper circuitry formation and disrupted in various neurodevelopmental disorders. Based on these findings we propose that miR-137- and its associated gene networks may constitute a gene-miR network to contribute to neuronal development and plasticity. Our results may provide clues for future research into drug targets related to miR-137 for cognitive dysfunction associated SZ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Aron Kos for technical assistance. The research of the authors is supported by grants from the “Donders Center for Neuroscience fellowship award of the Radboudumc” [to A.A. and N.N.K.]; the “FP7-Marie Curie International Reintegration Grant” [to A.A. grant number 276868 and N.N.K. grant number 277091]; the Jerome Lejeune Foundation [to N.N.K.] and GENCODYS, an EU FP7 large-scale integrating project grant [Grant number 241995] [to HvB].

References

1. Nadif Kasri N, Nakano-Kobayashi A, Van Aelst L. Rapid synthesis of the X-linked mental retardation protein OPHN1 mediates mGluR-dependent LTD through interaction with the endocytic machinery. *Neuron*. Oct 20; 2011 72(2):300–315. [PubMed: 22017989]
2. Nadif Kasri N, Nakano-Kobayashi A, Malinow R, Li B, Van Aelst L. The Rho-linked mental retardation protein oligophrenin-1 controls synapse maturation and plasticity by stabilizing AMPA receptors. *Genes & development*. Jun 1; 2009 23(11):1289–1302. [PubMed: 19487570]
3. Aschrafi A, Cunningham BA, Edelman GM, Vanderklish PW. The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain 8. *ProcNatlAcadSciUSA*. 2005; 102(6):2180–2185.
4. Kos A, Aschrafi A, Nadif Kasri N. The Multifarious Hippocampal Functions of MicroRNA-137. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*. Sep 22.2015
5. Gardiner E, Beveridge NJ, Wu JQ, Carr V, Scott RJ, Tooney PA, Cairns MJ. Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells. *Molecular psychiatry*. Jul; 2012 17(8):827–840. [PubMed: 21727898]
6. Xu B, Hsu PK, Stark KL, Karayiorgou M, Gogos JA. Derepression of a Neuronal Inhibitor due to miRNA Dysregulation in a Schizophrenia-Related Microdeletion. *Cell*. Jan 17; 2013 152(1-2):262–275. [PubMed: 23332760]
7. Guella I, Sequeira A, Rollins B, et al. Evidence of allelic imbalance in the schizophrenia susceptibility gene ZNF804A in human dorsolateral prefrontal cortex. *Schizophrenia research*. Jan; 2014 152(1):111–116. [PubMed: 24315717]
8. Kim AH, Parker EK, Williamson V, McMichael GO, Fanous AH, Vladimirov VI. Experimental validation of candidate schizophrenia gene ZNF804A as target for hsa-miR-137. *Schizophrenia research*. Oct; 2012 141(1):60–64. [PubMed: 22883350]
9. Kwon E, Wang W, Tsai LH. Validation of schizophrenia-associated genes CSMD1, C10orf26, CACNA1C and TCF4 as miR-137 targets. *Molecular psychiatry*. Jan; 2013 18(1):11–12. [PubMed: 22182936]
10. Ripke S, O'Dushlaine C, Chambert K, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nature genetics*. Oct; 2013 45(10):1150–1159. [PubMed: 23974872]
11. Schizophrenia Psychiatric Genome-Wide Association Study C. Genome-wide association study identifies five new schizophrenia loci. *Nature genetics*. Oct; 2011 43(10):969–976. [PubMed: 21926974]
12. Green MJ, Cairns MJ, Wu J, et al. Genome-wide supported variant MIR137 and severe negative symptoms predict membership of an impaired cognitive subtype of schizophrenia. *Molecular psychiatry*. Jul; 2013 18(7):774–780. [PubMed: 22733126]
13. Kuswanto CN, Sum MY, Qiu A, Sitoh YY, Liu J, Sim K. The impact of genome wide supported microRNA-137 (MIR137) risk variants on frontal and striatal white matter integrity, neurocognitive functioning, and negative symptoms in schizophrenia. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. Jul; 2015 168B(5):317–326.
14. Siegert S, Seo J, Kwon EJ, et al. The schizophrenia risk gene product miR-137 alters presynaptic plasticity. *Nature neuroscience*. May 25.2015
15. Willemsen MH, Valles A, Kirkels LA, et al. Chromosome 1p21.3 microdeletions comprising DPYD and MIR137 are associated with intellectual disability. *Journal of medical genetics*. Dec; 2011 48(12):810–818. [PubMed: 22003227]
16. Smrt RD, Szulwach KE, Pfeiffer RL, et al. MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. *Stem cells*. 2010; 28(6):1060–1070. [PubMed: 20506192]
17. Szulwach KE, Li X, Smrt RD, et al. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. *The Journal of cell biology*. Apr 5; 2010 189(1):127–141. [PubMed: 20368621]
18. Olde Loohuis NF, Ba W, Stoerchel PH, et al. MicroRNA-137 Controls AMPA-Receptor-Mediated Transmission and mGluR-Dependent LTD. *Cell reports*. Jun 30; 2015 11(12):1876–1884. [PubMed: 26095359]

19. Olde Loohuis NF, Kos A, Martens GJ, Van Bokhoven H, Nadif Kasri N, Aschrafi A. MicroRNA networks direct neuronal development and plasticity. *Cellular and molecular life sciences : CMLS*. Jan; 2012 69(1):89–102. [PubMed: 21833581]
20. van de V II, Gordebeke PM, Khoshab N, Tiesinga PH, Buitelaar JK, Kozicz T, Aschrafi A, Glennon JC. Long non-coding RNAs in neurodevelopmental disorders. *Front Mol Neurosci*. 2013; 6:53. [PubMed: 24415997]
21. Wright C, Turner JA, Calhoun VD, Perrone-Bizzozero N. Potential Impact of miR-137 and Its Targets in Schizophrenia. *Front Genet*. 2013; 4:58. [PubMed: 23637704]
22. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols*. 2009; 4(1):44–57. [PubMed: 19131956]
23. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome biology*. Jun 18, 2002 3(7):RESEARCH0034. [PubMed: 12184808]
24. Aschrafi A, Schwechter AD, Mameza MG, Natera-Naranjo O, Gioio AE, Kaplan BB. MicroRNA-338 regulates local cytochrome c oxidase IV mRNA levels and oxidative phosphorylation in the axons of sympathetic neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. Nov 19; 2008 28(47):12581–12590. [PubMed: 19020050]
25. Edbauer D, Neilson JR, Foster KA, et al. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron*. 2010; 65(3):373–384. [PubMed: 20159450]
26. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. *PLoS biology*. Nov. 2004 2(11):e363. [PubMed: 15502875]
27. Wang X. miRDB: a microRNA target prediction and functional annotation database with a wiki interface. *Rna Jun*. 2008; 14(6):1012–1017.
28. Wang X, El Naqa IM. Prediction of both conserved and nonconserved microRNA targets in animals. *Bioinformatics*. Feb 1; 2008 24(3):325–332. [PubMed: 18048393]
29. Dweep H, Sticht C, Pandey P, Gretz N. miRWalk--database: prediction of possible miRNA binding sites by “walking” the genes of three genomes. *Journal of biomedical informatics*. Oct; 2011 44(5):839–847. [PubMed: 21605702]
30. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005; 120(1):15–20. [PubMed: 15652477]
31. Helwak A, Kudla G, Dudnakova T, Tollervey D. Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. *Cell*. Apr 25; 2013 153(3):654–665. [PubMed: 23622248]
32. Lang B, Pu J, Hunter I, et al. Recurrent deletions of ULK4 in schizophrenia: a gene crucial for neurogenesis and neuronal motility. *Journal of cell science*. Feb 1; 2014 127(Pt 3):630–640. [PubMed: 24284070]
33. Crisafulli C, Drago A, Calabro M, Spina E, Serretti A. A molecular pathway analysis informs the genetic background at risk for schizophrenia. *Progress in neuro psychopharmacology & biological psychiatry*. Jun 3, 2015 59:21–30. [PubMed: 25554435]
34. Funk AJ, McCullumsmith RE, Haroutunian V, Meador-Woodruff JH. Abnormal activity of the MAPK- and cAMP-associated signaling pathways in frontal cortical areas in postmortem brain in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Mar; 2012 37(4):896–905. [PubMed: 22048463]
35. Kyosseva SV. Differential expression of mitogen-activated protein kinases and immediate early genes fos and jun in thalamus in schizophrenia. *Progress in neuro psychopharmacology & biological psychiatry*. Sep; 2004 28(6):997–1006. [PubMed: 15380860]
36. Kyosseva SV, Elbein AD, Griffin WS, Mrak RE, Lyon M, Karson CN. Mitogen-activated protein kinases in schizophrenia. *Biological psychiatry*. Sep 1; 1999 46(5):689–696. [PubMed: 10472421]
37. Yuan P, Zhou R, Wang Y, Li X, Li J, Chen G, Guitart X, Manji HK. Altered levels of extracellular signal-regulated kinase signaling proteins in postmortem frontal cortex of individuals with mood disorders and schizophrenia. *Journal of affective disorders*. Jul; 2010 124(1-2):164–169. [PubMed: 19913919]

38. Kyosseva SV, Elbein AD, Hutton TL, Griffin ST, Mrak RE, Sturner WQ, Karson CN. Increased levels of transcription factors Elk-1, cyclic adenosine monophosphate response element-binding protein, and activating transcription factor 2 in the cerebellar vermis of schizophrenic patients. *Archives of general psychiatry*. Jul; 2000 57(7):685–691. [PubMed: 10891039]
39. Todorova VK, Elbein AD, Kyosseva SV. Increased expression of c-Jun transcription factor in cerebellar vermis of patients with schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Aug; 2003 28(8):1506–1514. [PubMed: 12799614]
40. Pietersen CY, Mauney SA, Kim SS, et al. Molecular profiles of pyramidal neurons in the superior temporal cortex in schizophrenia. *J Neurogenet*. Mar-Jun; 2014 28(1-2):53–69. [PubMed: 24702465]
41. Lamprecht R. The actin cytoskeleton in memory formation. *Progress in neurobiology*. Jun. 2014 117:1–19. [PubMed: 24530292]
42. Wu X, Reddy DS. Integrins as receptor targets for neurological disorders. *Pharmacology & therapeutics*. Apr; 2012 134(1):68–81. [PubMed: 22233753]
43. Dityatev A, Schachner M, Sonderegger P. The dual role of the extracellular matrix in synaptic plasticity and homeostasis. *Nature reviews Neuroscience*. Nov; 2010 11(11):735–746. [PubMed: 20944663]
44. Evans AR, Euteneuer S, Chavez E, Mullen LM, Hui EE, Bhatia SN, Ryan AF. Laminin and fibronectin modulate inner ear spiral ganglion neurite outgrowth in an in vitro alternate choice assay. *Developmental neurobiology*. Nov; 2007 67(13):1721–1730. [PubMed: 17600813]
45. Gomez TM, Letourneau PC. Filopodia initiate choices made by sensory neuron growth cones at laminin/fibronectin borders in vitro. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. Oct; 1994 14(10):5959–5972. [PubMed: 7931556]
46. McKerracher L, Chamoux M, Arregui CO. Role of laminin and integrin interactions in growth cone guidance. *Molecular neurobiology*. Apr; 1996 12(2):95–116. [PubMed: 8818145]
47. Chan WL, Holstein-Rathlou NH, Yip KP. Integrin mobilizes intracellular Ca(2+) in renal vascular smooth muscle cells. *American journal of physiology Cell physiology*. Mar; 2001 280(3):C593–603. [PubMed: 11171579]
48. Gui P, Wu X, Ling S, et al. Integrin receptor activation triggers converging regulation of Cav1.2 calcium channels by c-Src and protein kinase A pathways. *The Journal of biological chemistry*. May 19; 2006 281(20):14015–14025. [PubMed: 16554304]
49. Wu X, Davis GE, Meininger GA, Wilson E, Davis MJ. Regulation of the L-type calcium channel by alpha 5beta 1 integrin requires signaling between focal adhesion proteins. *The Journal of biological chemistry*. Aug 10; 2001 276(32):30285–30292. [PubMed: 11382763]
50. Chan CS, Weeber EJ, Zong L, Fuchs E, Sweatt JD, Davis RL. Beta 1-integrins are required for hippocampal AMPA receptor-dependent synaptic transmission, synaptic plasticity, and working memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. Jan 4; 2006 26(1):223–232. [PubMed: 16399691]
51. Fan Y, Abrahamsen G, Mills R, et al. Focal adhesion dynamics are altered in schizophrenia. *Biological psychiatry*. Sep 15; 2013 74(6):418–426. [PubMed: 23482246]
52. Gardel ML, Schneider IC, Aratyn-Schaus Y, Waterman CM. Mechanical integration of actin and adhesion dynamics in cell migration. *Annual review of cell and developmental biology*. 2010; 26:315–333.
53. Spillane M, Gallo G. Involvement of Rho-family GTPases in axon branching. *Small GTPases*. 2014; 5:e27974. [PubMed: 24936971]
54. Collins AL, Kim Y, Bloom RJ, Kelada SN, Sethupathy P, Sullivan PF. Transcriptional targets of the schizophrenia risk gene MIR137. *Transl Psychiatry*. 2014; 4:e404. [PubMed: 24984191]
55. Hill MJ, Donocik JG, Nuamah RA, Mein CA, Sainz-Fuertes R, Bray NJ. Transcriptional consequences of schizophrenia candidate miR-137 manipulation in human neural progenitor cells. *Schizophr Res*. Mar; 2014 153(1-3):225–230. [PubMed: 24556472]
56. Wang X, Li Y, Xu X, Wang YH. Toward a system-level understanding of microRNA pathway via mathematical modeling. *Biosystems*. Apr; 2010 100(1):31–38. [PubMed: 20005918]

57. Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D. Specific effects of microRNAs on the plant transcriptome. *Developmental cell*. Apr; 2005 8(4):517–527. [PubMed: 15809034]
58. Baulcombe D. RNA silencing in plants. *Nature*. 2004; 431(7006):356–363. [PubMed: 15372043]
59. Liang H, Li WH. MicroRNA regulation of human protein protein interaction network. *Rna Sep*. 2007; 13(9):1402–1408.
60. Fukao A, Mishima Y, Takizawa N, et al. MicroRNAs trigger dissociation of eIF4AI and eIF4AII from target mRNAs in humans. *Molecular cell*. Oct 2; 2014 56(1):79–89. [PubMed: 25280105]
61. Wright C, Calhoun VD, Ehrlich S, Wang L, Turner JA, Bizzozero NI. Meta gene set enrichment analyses link miR-137-regulated pathways with schizophrenia risk. *Frontiers in genetics*. 2015; 6:147. [PubMed: 25941532]
62. Shi W, Du J, Qi Y, et al. Aberrant expression of serum miRNAs in schizophrenia. *Journal of psychiatric research*. Feb; 2012 46(2):198–204. [PubMed: 22094284]
63. Shi W, Du J, Qi Y, et al. Aberrant expression of serum miRNAs in schizophrenia. *Journal of psychiatric research*. Feb; 2012 46(2):198–204. [PubMed: 22094284]
64. Sun XY, Zhang J, Niu W, et al. A preliminary analysis of microRNA as potential clinical biomarker for schizophrenia. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. Apr; 2015 168B(3):170–178.
65. Fan HM, Sun XY, Niu W, et al. Altered microRNA Expression in Peripheral Blood Mononuclear Cells from Young Patients with Schizophrenia. *Journal of molecular neuroscience : MN*. Jul; 2015 56(3):562–571. [PubMed: 25665552]
66. Yu HC, Wu J, Zhang HX, et al. Alterations of miR-132 are novel diagnostic biomarkers in peripheral blood of schizophrenia patients. *Progress in neuro psychopharmacology & biological psychiatry*. Dec 3.2015 63:23–29. [PubMed: 25985888]
67. Witwer KW. Circulating microRNA biomarker studies: pitfalls and potential solutions. *Clinical chemistry*. Jan; 2015 61(1):56–63. [PubMed: 25391989]
68. Magen I, Hornstein E. Oligonucleotide-based therapy for neurodegenerative diseases. *Brain research*. Oct 10.2014 1584:116–128. [PubMed: 24727531]
69. Duygu B, de Windt LJ, da Costa Martins PA. Targeting microRNAs in heart failure. *Trends in cardiovascular medicine*. May 30.2015
70. Miller TM, Pestronk A, David W, et al. An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. *The Lancet Neurology*. May; 2013 12(5):435–442. [PubMed: 23541756]
71. Janssen HL, Reesink HW, Lawitz EJ, et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med*. May 2; 2013 368(18):1685–1694. [PubMed: 23534542]

Highlights

- miR-137 acts as a gene network hub during synaptic development and physiology.
- Many target genes are bidirectionally upregulated or repressed by miR-137.
- miR-137 targets genes function in long-term potentiation and axonal guidance.
- Intracellular signaling pathways MAPK-ERK, JAK-STAT, RhoA and Pi3K are downstream of miR-137.

Fig. 1. RNA-sequencing analysis of miR-137-affected genes in primary rat hippocampal neurons

A) Volcano plot showing the distribution of gene expression in sponge-miR-137 infected hippocampal neurons. Green dots depict differentially expressed genes showing a fold change above 1.2 and a significant adjusted P-value ($P < 0.05$) following Benjamini and Hochberg correction for multiple testing.

B) Volcano plot showing the distribution of gene expression in neurons overexpressing miR-137. Green dots represent differentially expressed genes showing a fold change above 1.2 and a significant adjusted P-value ($P < 0.05$).

C) Venn diagrams presenting the number of transcripts significantly upregulated in sponge-miR-137 (green circles) and downregulated in oe-miR-137 (purple circles) infected neurons. 500 transcripts were bidirectionally regulated by sponge-miR-137 and oe-miR-137. The fraction of bidirectionally miR-137-regulated transcripts with miR-137 3'-UTR binding sites is shown in brackets. The Venn diagram displayed in the lower panel represents the subset of transcripts downregulated in sponge-miR-137 and upregulated in oe-miR-137 neurons, in which a total of 27 transcripts was bidirectionally regulated.

D) A selection of genes with an RPKM above 1 and a fold change above 1.2 was validated by qPCR. The black line represents the relative expression value in the control condition, which was set to 1. Grey bars represent the sponge-miR-137 condition and black bars miR-137 overexpression. Gene expression was normalized to U6 and beta-actin. Bars represent mean $\pm$ SEM. # $P < 0.1$; * $P < 0.05$; ** $P < 0.01$ by one-way ANOVA.

Fig. 2. miR-137 affected genes functioning in neurodevelopmental processes

Genes bidirectionally regulated by miR-137 in hippocampal neurons are involved in proliferation, migration and differentiation of neurons as well as glial cells. Genes expressed in both neurons and glial cells are depicted in red, neuron-specific genes in black, glial cell specific genes in green and genes of which no cell-specific information is available in blue. Additional detailed information on the genes is provided in Suppl. Table 3A.

Fig. 3. miR-137 affected genes functioning in neuronal maturation processes

Bidirectionally regulated genes are involved in neurite outgrowth, dendritic tree complexity, spine and synapse functionality and axonal outgrowth and guidance. Genes expressed in both neurons and glial cells are depicted in red, neuron-specific genes in black, glial cell specific genes in green and genes of which no cell-specific information is available in blue. Additional detailed information on the genes is provided in Suppl. Table 3B.

Fig. 4. miR-137 affected genes functioning in cell maintenance

Bidirectionally regulated genes are involved apoptosis, cell survival and cell protection from physical damage. Genes expressed in both neurons and glial cells are depicted in red, neuron-specific genes in black, glial cell specific genes in green and genes of which no cell-specific information is available in blue. Additional detailed information on the genes is provided in Suppl. Table 3C.

Fig. 5. miR-137 affected genes functioning in intracellular signaling
Genes involved in the major intracellular signaling pathways MAPK-ERK, JAK-STAT, RhoA and Pi3K signaling. Genes expressed in both neurons and glial cells are depicted in red, neuron-specific genes in black, glial cell specific genes in green and genes of which no cell-specific information is available in blue. Additional detailed information on the genes is provided in Suppl. Table 3D.