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Analysis of miR-137 expression and rs1625579 in dorsolateral prefrontal cortex

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that act as potent regulators of gene expression. A recent GWAS reported the rs1625579 SNP, located downstream of miR-137, as the strongest new association with schizophrenia [Ripke S, Sanders AR, Kendler KS, Levinson DF, Sklar P, Holmans PA, et al. Genome-wide association study identifies five new schizophrenia loci. Nat Genet 2011;43:969–76.]. Prior to this GWAS finding, a schizophrenia imaging-genetic study found miR-137 target genes significantly enriched for association with activation in the dorsolateral prefrontal cortex (DLPFC) [Potkin SG, Macciardi F, Guffanti G, Fallon JH, Wang Q, Turner JA, et al. Identifying gene regulatory networks in schizophrenia. Neuroimage 2010;53:839–47.].

We investigated the expression levels of miR-137 and three candidate target genes (ZNF804A, CACNA1C, TCF4) in the DLPFC of postmortem brain tissue from 2 independent cohorts: (1) 26 subjects (10 control (CTR), 7 schizophrenia (SZ), 9 bipolar disorder (BD)) collected at the UCI brain bank; and (2) 99 subjects (33 CTR, 35 SZ, 31 BD) obtained from the Stanley Medical Research Institute (SMRI). MiR-137 expression in the DLPFC did not differ between diagnoses. We also explored the relationship between rs1625579 genotypes and miR-137 expression. Significantly lower miR-137 expression levels were observed in the homozygous TT subjects compared to TG and GG subjects in the control group (30% decrease, p-value = 0.03). Moreover, reduced miR-137 levels in TT subjects corresponded to increased levels of the miR-137 target gene TCF4. The miR-137 expression pattern in 9 brain regions was significant for regional effect (ANOVA p-value = 1.83E-12), with amygdala and hippocampus having the highest miR-137 expression level. In conclusion, decreased miR-137 expression is associated with the SZ risk allele of rs1625579, and potential regulation of TCF4, another SZ candidate gene. This study offers additional support for involvement of miR-137 and downstream targets as mechanisms of risk for psychiatric disorders.

1. Introduction

Family, twin and adoption studies have shown that both bipolar disorder (BD) and schizophrenia (SZ) have strong genetic components (Shih et al., 2004), and growing evidence suggests some overlapping genetic risk and expression profiles between SZ and BD (Craddock et al., 2006; Lichtenstein et al., 2009; Potkin et al., 2010; Purcell et al., 2009; Ripke et al., 2011; Shao and Vawter, 2008). Several robust and replicable genetic findings have been reported for both BD and SZ (Sullivan et al., 2012), and the obtained results are consistent with a combined contribution of both rare and common variants to disease susceptibility (McCarthy et al., 2008). The largest genome-wide association study (GWAS) for SZ

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to date, performed by The Psychiatric Genomics Consortium (PGC) on over 40,000 individuals from 17 separate studies, identified rs1625579 as the strongest new association with SZ (Ripke et al., 2011). This SNP is over 100 kb from any RefSeq protein-coding gene, but is located within an intron of the MIR137 host gene (MIR137HG), the putative primary transcript for miR-137.

An imaging-genetic study originally found miR-137 to be one of three miRNAs with target genes significantly enriched for association with activation in the DLPFC of individuals with SZ (Potkin et al., 2010). Additionally, putative miR-137 target genes are significantly enriched for association with SZ and/or BD in the PGC study (Ripke et al., 2011), and five of these associated genes (ZNF804A, CSMD1, C10orf26, TCF4 and CACNA1C) have been also validated as miR-137 targets by in-vitro methods (Kim et al., 2012; Kwon et al., 2011).

MicroRNAs (miRNAs) are a large family of small non-coding RNAs that negatively regulate the expression of more than 60% of protein-coding genes (Friedman et al., 2009). The nervous system has the broadest spectrum of miRNA expression of all human tissues. Approximately 70% of known miRNAs are expressed in the brain, and many of them are specific to neurons (Krichevsky et al., 2003). MiRNAs have already been identified as essential factors in regulating brain development and function (Coolen and Bally-Cuif, 2009), and miRNAs dysregulation in postmortem brain samples from individuals with SZ or BD has been reported in several independent studies (Banigan et al., 2013; Beveridge et al., 2010; Beveridge et al., 2008; Kim et al., 2010; Mellios et al., 2012, 2009; Miller et al., 2012; Moreau et al., 2011; Perkins et al., 2007; Santarelli et al., 2011; Zhu et al., 2009). Moreover, miR-137 is a key regulator of neuronal development with roles in adult neurogenesis, dendritic development (Szulwach et al., 2010), and neuronal maturation (Smrt et al., 2010), and is highly expressed at synapses in the cortex and hippocampus (Willemsen et al., 2011). Thus, miRNAs could play a crucial role in the networks of regulatory mechanisms involved in SZ and BD.

Additional studies are needed to elucidate the role of miR-137 in SZ and BD susceptibility. In this study, we investigated the possible involvement of miR-137 in SZ and BD by evaluating the expression of miR-137 and of three of its predicted target genes (ZNF804A, CACNA1C and TCF4) in postmortem brains of SZ and BD subjects. Moreover, we also explored the relationship between rs1625579 genotypes and miR-137 expression.

2. Methods

2.1. Postmortem brain samples

Two sets of brain tissue samples, obtained from the University of California, Irvine Brain Bank (UCI BB) and the Stanley Medical Research Institute (SMRI, http://www.stanleyresearch.org), were used in this study.

2.1.1. UCI BB cohort

This cohort includes postmortem brain tissues of 26 subjects (10 controls (CTR), 7 schizophrenia (SZ), and 9 bipolar disorder (BD) subjects) from the following regions: anterior cingulate cortex (ACC), amygdala (AMY), caudate nucleus (CAUN), cerebellum (CBL), dorsolateral prefrontal cortex (DLPFC), hippocampus (HIPP), nucleus accumbens (NACC), orbitofrontal cortex (OFC), putamen (PUT), and thalamus (THAL).

Written informed consent was obtained from the next of kin for each subject and the study was reviewed and approved by UCI Institutional Review Board. All subjects went through an extensive review of multiple sources of information including the medical examiner's conclusions, coroner's investigation, medical and psychiatric records, toxicology results, interviews of the decedents' next-of-kin, and gross neuropathology examination to rule out as much as possible any neurodegenerative disorder. All subjects were clinically characterized with the psychological autopsy method and died suddenly without prolonged agonal state. The human brain dissection and freezing protocol is described in detail elsewhere (Jones et al., 1992).

RNA was prepared from 80 to 100 mg of frozen tissue samples, with Omni Prep Multi-Sample Homogenizer (Omni International, Kennesaw, GA). Total RNA isolation was performed with TRIZOL™ reagent (Invitrogen, Carlsbad, CA), following the manufacturer's instructions. RNA was quantified by OD260/280 with a UV spectrophotometer and treated with RNase-free DNase using the RNeasy MinElute® columns (Qiagen, Valencia, CA). The quality of the total RNA was finally evaluated using the Agilent 2100 Bioanalyser RNA Chip (Santa Clara, CA). Genomic DNA was extracted from tissues using the DNeasy Blood & Tissue Kits (Qiagen), following the manufacturer's instructions.

2.1.2. SMRI cohort

The SMRI Array Collection is a collection of postmortem brains from individuals with SZ ($n = 35$), with BD ($n = 34$), and psychiatrically normal controls ($n = 35$). For detailed sample information see Supplementary Methods. DNA and RNA from the DLPFC were available respectively for 82 and 99 individuals in the collection. Several demographic and clinical variables, i.e. gender, age, postmortem interval (PMI), brain pH, RNA Integrity Number (RIN), and lifetime antipsychotic exposure in fluphenazine mg equivalents were evaluated as potential covariates. Similar covariates were used in the analysis of the UCI BB cohort. Further information about the SMRI Collection is available at http://www.stanleyresearch.org/programs/brain_collection.asp.

2.2. Genotyping of rs1625579 by real-time PCR

TaqMan probes and primers were designed and synthesized by Applied Biosystems (Foster City, CA). PCRs were performed on 10 ng of genomic DNA in a 5 μL final volume using the TaqMan Genotyping Master Mix and TaqMan SNP genotyping assay on a 7900HT real-time sequence detection system (Applied Biosystems). The assay was validated in house by Sanger sequencing random subjects, and in all cases were concordant to the TaqMan assay results.

2.3. MiR-137 expression

The miR-137 expression level was evaluated by real-time qPCR, using the following procedure. In brief, miRNAs were reverse transcribed (RT) starting from 500 ng of total RNA, using the miRNA First Strand Synthesis kit (Agilent Technologies), according to the manufacturer's instructions. An aliquot of the RT reaction was then used as template in a standard real-time RT-PCR amplification, using Power SybrGreen master mix (Applied Biosystems), the universal reverse primer (Agilent Technologies) and a miRNA-specific forward primer (Supplementary Table 1). Real-time RT-PCR conditions were set up to favor the amplification of only the mature miRNAs and a dissociation curve analysis was performed for every reaction to confirm the presence of a single specific amplification product.

Real-time RT–PCR assays were performed in triplicate on a 7900HT real-time sequence detection system (Applied Biosystems). Differences in the miRNA expression levels were quantified by the ΔΔCT method using the small nuclear RNA (snRNA) U6 for internal normalization (see Supplementary Table 1 for primer sequences), also run in triplicate (Livak and Schmittgen, 2001). The snRNA U6

was selected since it's one of the sn/snoRNA that has the highest abundance and least variability across normal tissues (Wong et al., 2007).

An ANOVA model was used to test the significance of miR-137 differential expression between SZ, BD, and CTR subjects, and to analyze the relationship between rs1625579 genotypes and miR-137 expression. The potential confounding effects of age, gender, RIN, and pH in the analysis of miR-137 expression were assessed by correlation. Considering the two cohorts were from different brain banks, in order to adjust for the confounding effect of site (p-value = 0.013) the residuals of the miR-137 expression levels were obtained after regression for the two sites and used in all joint cohort analyses.

2.4. MiR-137 target genes validation

The expression levels of ZNF804A, CACNA1C and TCF4 were evaluated by real-time qPCR.

Total RNA (500 ng) was reverse transcribed with oligo (dT) primers and MultiScribe Reverse Transcriptase, using the TaqMan Gold RT-PCR Kit (Invitrogen), according to the manufacturer's instructions. RT-PCRs were carried out under standard conditions using the Power SYBR® Green Master Mix on a on a 7900HT real-time sequence detection system (Applied Biosystems). All reactions were performed in triplicate and data normalized using three housekeeping genes (GAPDH, HPRT1 and SDHA) and the software GeNorm (Vandesompele et al., 2002). Primer sequences are listed in Supplementary Table 1. The potential confounding effects of age, sex, RIN, and pH in the analysis of miR-137 target genes expression were assessed by correlation. In order to adjust for the confounding effect of site, the residuals of ZNF804A, CACNA1C and TCF4 expression levels were obtained after regression for the two sites and used in all joint cohort analyses. Correlation between miR-137 and the mRNA levels of the three miR-137 target genes was tested with Pearson's product moment correlation.

An ANOVA model was used to analyze the relationship between rs1625579 genotypes and the ZNF804A, CACNA1C and TCF4 mRNA expression levels.

3. Results

The expression levels of miR-137 and three of its predicted target genes were evaluated in the DLPFC of SZ, BD and CTR subjects. The relationship between rs1625579 genotypes and miR-137 expression levels was also analyzed.

3.1. Cohort demographic and clinical characteristics

Demographic and clinical characteristics of the postmortem brain samples from both cohorts are listed in Table 1. Brain pH was the only variable that was significantly different among diagnostic groups in both cohorts. However, the pH does not correlate with miR-137 or target genes expression. In the UCI cohort only, the RIN was significantly higher in the BD samples, and a significant correlation (p-value<0.001) between the RIN and miR-137 expression was found, therefore we included RIN as a covariate for the analysis of the UCI cohort. None of the covariates (pH, age, RIN, PMI) showed correlations with miR-137 or target genes expression in the two cohorts combined together, or in the SMRI; therefore these covariates were not included in the ANOVA models. No effects were found for the lifetime exposure to antipsychotic medication, fluphenazine equivalents, available only for the SMRI cohort.

3.2. Effect of diagnosis on expression of miR-137 in DLPFC

The miR-137 expression levels were evaluated by real-time PCR in the DLPFC of postmortem brains from 2 independent cohorts: (i) 26 subjects (10 CTR, 7 SZ, 9 BD) collected at the UCI BB; and (ii) 99 subjects (33 CTR, 35 SZ, 31 BD) obtained from the SMRI Array collection.

No significant differences in miR-137 expression were observed in BD or SZ subjects, as compared to controls and normalized to snRNA U6, in the UCI and the SMRI cohort (Supplementary Table 2). Additionally, the combined analysis performed on the two cohorts together showed no significant differences in the miR-137 expression on the basis of diagnostic classification (p-value = 0.48) (Fig. 1).

3.3. Effect of rs1625579 genotype on miR-137 expression

To investigate the possible association between the genome-wide significant SZ associated rs1625579 SNP and miR-137 expression, we genotyped this SNP in all the available samples from the two cohorts. The rs1625579 genotypes (TT, TG, and GG) were obtained for a total of 108 samples, and genotype distributions were in line with Hardy-Weinberg expectations. As expected given the really low frequency of the rs1625579 SNP, we only found 2 homozygous GG subjects. Therefore, these two subjects were grouped with the heterozygous TG subjects. All analyses of genotype effects were conducted on both cohorts together.

A non-significant reduction of miR-137 levels was observed in the homozygous TT subjects, as compared to TG and GG subjects (0.87 fold decrease, p-value = 0.23). Although this difference did not achieve statistical significance by ANOVA, when controls only were analyzed, the miR-137 expression was significantly lower for the homozygous TT subjects (0.70 fold decrease, p-value = 0.034) (Fig. 2).

3.4. MiR-137 target genes

In addition to be associated with SZ and/or BD, ZNF804A, CACNA1C and TCF4 have been predicted to be miR-137 target genes,

Table 1
Demographic and clinical characteristics of the postmortem brain samples used in this study.

	UCI (N = 26)			SMRI (N = 99)		
	CTR	SZ	BD	CTR	SZ	BD
N of subjects	10	7	9	33	35	31
Female (%)	30.0	42.9	44.4	25.7	27.3	51.6
Age (±SD)	48.0 ± 13.0	46.1 ± 10.0	51.7 ± 13.8	43.6 ± 7.6	42.6 ± 8.5	44.9 ± 11.0
PMI (±SD)	20.5 ± 8.3	22.3 ± 7.1	22.6 ± 7.6	29.4 ± 13.2	31.4 ± 15.5	36.6 ± 18.1
pH (±SD)	6.15 ± 0.2	6.53 ± 0.4[a]	6.6 ± 0.4[a]	6.6 ± 0.3	6.5 ± 0.2[a]	6.5 ± 0.3[a]
RIN (±SD)	5.7 ± 1.0	5.1 ± 1.3	6.9 ± 0.9[a]	7.6 ± 0.9	7.6 ± 0.7	7.5 ± 0.9
Age of onset (±SD)	–	23.6 ± 4.3	30.8 ± 9.5	–	21.3 ± 6.1	24.8 ± 8.9
Smoking (%)	NA	100	100	NA	85.2	70
Suicide (%)	0	42.8	0	0	20.0	45.2

[a] p-value <0.05 compared to control group, NA, not available for all subjects.

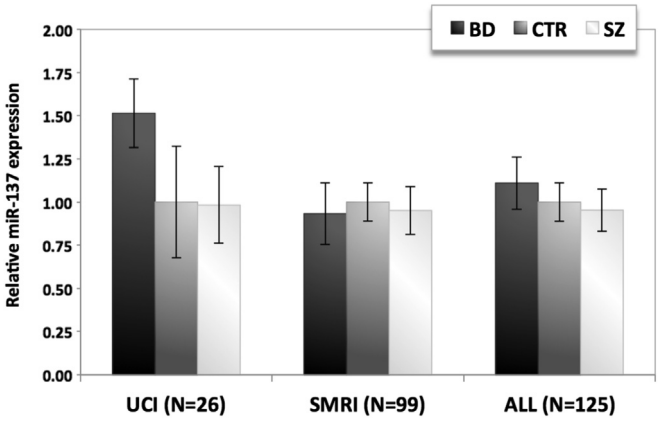

Fig. 1. MiR-137 expression in the DLPFC of SZ, BD and CTR subjects. Variations in miR-137 expression levels were quantified using the ΔΔCT method; in all experiments, the snRNA U6 was used as reference. The results for the UCI, SMRI, and for the two cohorts combined (ALL) are shown. Level of expression for controls was set at 1; bars represent group mean ± SEM. Each individual sample was run in triplicate.

and have also been validated by in-vitro methods (Kim et al., 2012; Kwon et al., 2011). To assess whether miR-137 modulates the expression of these three genes, we measured their transcript levels in the DLPFC samples by qPCR. MiR-137 expression levels did not show any significant correlation with the mRNA levels of any of these genes.

Next, we investigated if reduced levels of miR-137 in TT subjects corresponded to an increased level of ZNF804A, CACNA1C and TCF4 mRNAs. Only TCF4 was up-regulated in subjects homozygous for the T-risk allele, as compared to TG and GG subjects, both analyzing the three diagnostic groups together (fold increase 1.17, p-value = 0.052) or in the control group only (fold increase 1.33, p-value = 0.079) (Fig. 3).

3.5. MiR-137 expression regional variation

In order to analyze whether outlier values in the DLPFC might affect the miR-137 expression pattern in different brain regions, we measured its expression across 10 brain areas in 9 subjects belonging to the UCI cohort. The 9 subjects (3 for each diagnostic group) were selected for having low, medium and high miR-137 expression levels in the initial DLPFC assay. Levels of expression were therefore analyzed in the following additional regions: anterior cingulate cortex (ACC), amygdala (AMY), caudate nucleus (CAUN), cerebellum (CBL), hippocampus (HIPP), nucleus accumbens (NACC), orbitofrontal cortex (OFC), putamen (PUT), and thalamus (THAL).

The cerebellum had extremely low levels of miR-137 (1000 fold less compared to the median of other regions), and was not included in further analysis. As shown in Fig. 4A, in the 9 analyzed regions, miR-137 expression levels are significantly different across brain regions (ANOVA p-value: 1.83E-12), but not among different subjects (p-value = 0.60) or by diagnosis (p-value = 0.53). The highest miR-137 expression levels were observed in AMY, HIPP, NACC and OFC (Fig. 4A).

Next, we measured the miR-137 expression in all 26 UCI subjects in both AMY and HIPP, and compared to DLPFC. This experiment confirmed that miR-137 is particularly abundant in the AMY, with a 3.03 and 2.00 fold-increased expression as compared to the DLPFC and to the HIPP, respectively (Fig. 4B). Again, the average miR-137 expression level across these three brain regions was not significantly different between subjects, in agreement with the findings observed in the 10 regions studied above. In both AMY and HIPP, no significant changes in miR-137 expression were observed on the basis of diagnostic classification (data not shown) and a formal analysis of genotype effects was not conducted because of small number of minor allele subjects.

4. Discussion

Prior transcriptomic, proteomic, and genetic studies suggest that SZ is a complex disorder involving multiple pathways (Sullivan, 2012). Recently, a plausible network of genes involving miR-137 in SZ has emerged. Initial evidence supporting this miR-137 pathway came from the genome-wide significant association of the rs1625579 SNP with SZ (Ripke et al., 2011). Other studies also observed an enrichment for association with SZ of miR-137 predicted target genes (Potkin et al., 2010; Ripke et al., 2011).

However, to date there have been no studies investigating the molecular basis underlying this association. This lack may be

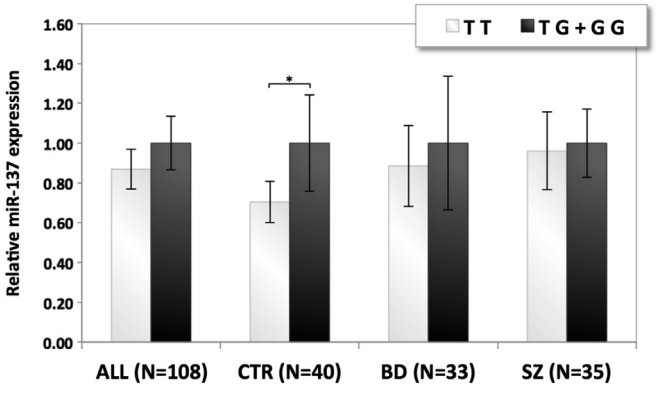

Fig. 2. Association between rs1625579 genotypes and miR-137 expression in the DLPFC. Variations in miR-137 expression levels between subjects with different rs1625579 genotypes were quantified using the ΔΔCT method; in all experiments, the snRNA U6 was used as reference. The differential expression by genotype analyses have been performed only on the UCI and SMRI cohorts combined, either on all subjects together (ALL) or grouped on the basis of the diagnosis. The miR-137 expression is significantly lower in the homozygous TT subjects compared to TG and GG subjects in the control group only (30% decrease, p-value = 0.034). Level of expression for non-risk allele subjects (TG + GG) was set at 1; bars represent group mean ± SEM. Each individual sample was run in triplicate. *p-value <0.05 (analysis of variance).

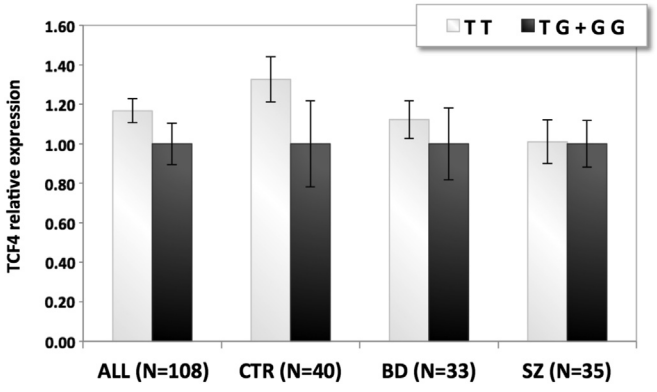

Fig. 3. Association between rs1625579 genotypes and TCF4 expression levels in the DLPFC. Variations in the TCF4 expression levels between subjects with different rs1625579 genotypes were quantified using the ΔΔCT method; in all experiments, the geometric mean of three housekeeping genes (GAPDH, HPRT1 and SDHA) was used as reference. The differential expression analyses have been performed only on two cohorts together, either on all subjects together (ALL), or grouped on the basis of the diagnosis. Level of expression for SNP rs1625579 TG + GG subjects was set at 1; bars represent group mean ± SEM. Each individual sample was run in triplicate.

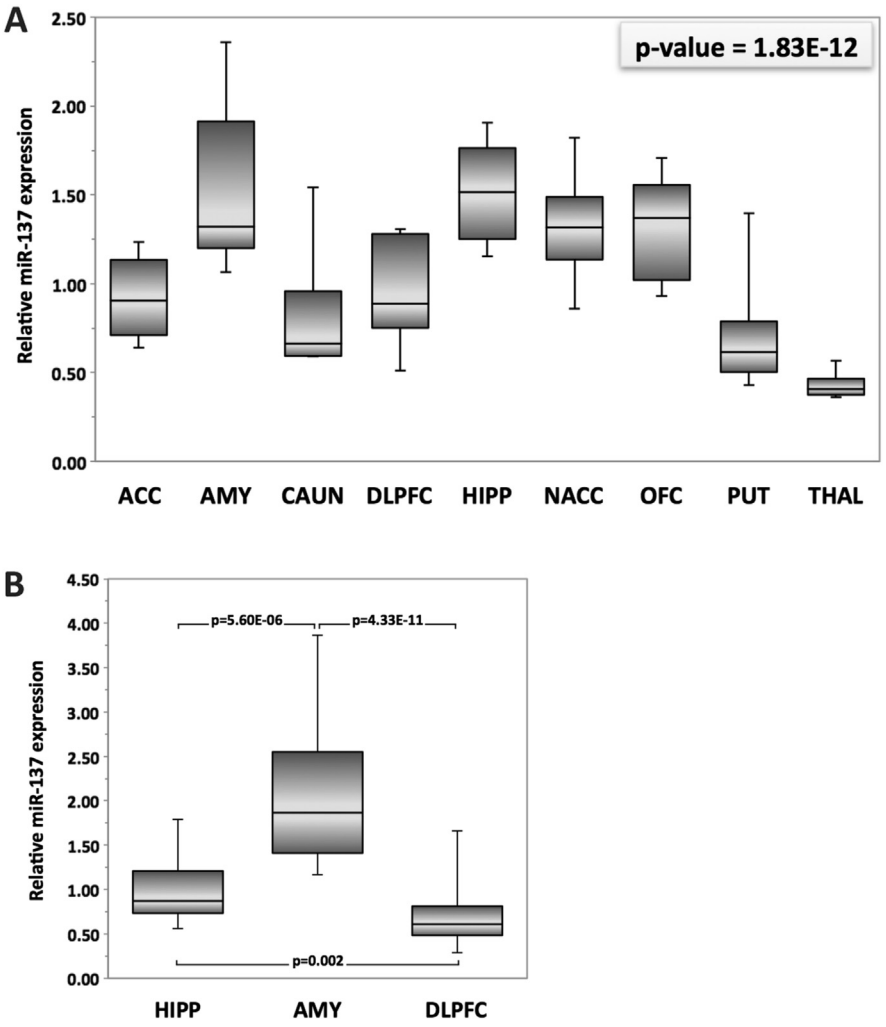

Fig. 4. Box-and-whisker plots representing the expression of miR-137 expression in different brain areas. (A) The miR-137 expression levels were measured across 9 brain regions in 9 subjects belonging to the UCI cohort (3 for each diagnostic group). MiR-137 expression is significantly different across brain regions (ANOVA *p*-value: 1.83E-12), but not among different subjects. (B) MiR-137 expression was measured in the DLPFC, AMY and HIPP in 26 UCI subjects. MiR-137 is particularly abundant in the AMY, with a 3.03 and 2.00-fold increased in the expression as compared to the DLPFC and to the HIPP, respectively. MiR-137 expression levels were obtained by the ΔΔCT method, the snRNA U6 was used as reference, and the median level of the normalized miR-137 expression across all brain regions was set at 1. Boxes represent the 25th to the 75th percentile, and the 50th percentile (median) is denoted by a horizontal line in the box. The whiskers are drawn down to the 10th percentile and up to the 90th.

explained as the result of the well-known bias toward publishing positive results. In the present study, no significant differences in miR-137 expression were observed between diagnoses. This finding is consistent with prior reports as miR-137 expression has not been reported to be dysregulated in any of the several studies investigating miRNAs expression levels in SZ and BD brains (Banigan et al., 2013; Beveridge et al., 2010, 2008; Kim et al., 2010; Mellios et al., 2012, 2009; Miller et al., 2012; Moreau et al., 2011; Perkins et al., 2007; Santarelli et al., 2011; Zhu et al., 2009) (see Beveridge and Cairns (2012) and Mellios and Sur (2012) for review).

Despite the lack of differences in miR-137 expression among BD, SZ, and CTR subjects, a post hoc analysis suggested association of miR-137 expression in the DLPFC with rs1625579 genotypes. In this post hoc analysis significantly lower expression levels of miR-137 were observed in control subjects homozygous for the T-risk allele of the SZ associated rs1625579 SNP, compared to TG and GG subjects. This genetic effect on miR-137 expression levels was only present in the control group, suggesting that other factors might play a bigger role in expression regulation in cases. It is tempting to speculate that for a variety of reasons (e.g. epigenetic, drug, or environmental changes) the genetic regulation of miR-137 expression is attenuated in patients. It is unlikely that the rs1625579 SNP is the functional variant influencing miR-137 expression, since it is located more than 8 kb downstream of the pre-miR-137 sequence. However, this SNP is within a 25-kb haplotype block containing the pre-miR-137 sequence, and therefore, it is possible that the GWAS-associated SNP is tagging another variant with a functional effect on the miR-137 expression or stability.

We focused our attention on miR-137 expression, although, there is a considerable distance between the rs1625579 SNP and the pre-miR-137 sequence, and there is a second miRNA, miR-2682, located less than 719 bp from miR-137. MiR-137 seems to be the best candidate gene present in the region tagged by rs1625579, because of the known involvement of miR-137 in neurodevelopment (Smrt et al., 2010; Szulwach et al., 2010), and the enrichment of miR-137 target genes significantly associated with activation in the DLPFC (Potkin et al., 2010). Finally, the miR-137 risk allele is associated with a cluster of predominantly negative symptoms (Cummings et al., 2012).

It is noteworthy to mention that a recent paper by Xu et al. (Xu et al., 2012) reported two de novo mutations in the DPYD gene. The two mutations were found in two family trios with SZ of Afrikaner and Caucasian Northern European origin, respectively. DPYD is located 124 kb from the rs1625579 SNP, within a haplotype block that extends from the 5′ exons of the gene to the associated SNP also including miR-137. Therefore the association of SZ with rs1625579 might also reflect the presence of another disease-associated variant in DPYD.

Consistent with the miR-137 network, we also found that reduction in brain levels of miR-137 in TT subjects corresponds to increased TCF4 mRNA levels, supporting the previous data from in-vitro studies, which suggested TCF4 as a miR-137 target gene (Kwon et al., 2011). This finding is relevant because TCF4 is one of a handful of highly replicated SZ loci (Ripke et al., 2011; Stefansson et al., 2009; Steinberg et al., 2011), and has also been associated with BD (Del-Favero et al., 2002). TCF4 is highly expressed in the brain, and it has higher expression in neocortex and hippocampus, brain areas that have been implicated with SZ (Navarrete et al., 2012). Variations in neurocognition in SZ are also associated with TCF4 (Lennertz et al., 2011; Quednow et al., 2011; Zhu et al., 2012). Moreover, altered TCF4 expression levels in SZ subjects in brain (increased levels) (Mudge et al., 2008) and blood (decreased levels) (Navarrete et al., 2012) have been reported. However, three other postmortem brain studies found no change in TCF4 expression (Barnes et al., 2011; Maycox et al., 2009; Narayan et al., 2008), as well as in our expression analysis we did not find any difference in TCF4 levels across different diagnostic groups.

It is interesting that miR-137 has been shown to be associated with brain activation across multiple regions, amygdala, pre/post central gyrus, and medial frontal gyrus (Whalley et al., 2012), consistent with our findings on the miR-137 expression pattern in brain. In our survey, miR-137 expression is significantly different across 10 brain regions, with the amygdala and hippocampus having the highest miR-137 expression levels. Moreover, we replicate the prior findings (Willemsen et al., 2011) of barely detectable levels of miR-137 in the cerebellum, using the anterior lobe of the cerebellum cortex, indicating that miR-137 is not ubiquitously expressed in all the brain, and suggesting the existence of a brain region-specific regulation mechanism.

Potential limitations of the present postmortem study include the sample size, and the unavoidable site-to-site variability in postmortem brain collections. A power analysis based on a moderate effect size of 0.5, as the one observed in this study, showed that a sample size of 210 subjects total (105/group) would be required to have a 95% power to replicate. Therefore, our findings could be statistically stronger with a larger sample size. Possible confounding variables, such as age, pH, RIN, gender, and fluphenazine lifetime equivalents, known to influence gene expression (Atz et al., 2007; Li et al., 2007), appeared not associated with miR-137 expression. Other potential sources of variability among subjects, involving smoking history, therapeutic medications, and drugs of abuse, might influence gene expression levels. SZ is thought to be a neurodevelopmental disorder involving disturbances in synaptic connection and brain wiring (Harrison and Weinberger, 2005). Finding the potential pathophysiology will require a developmental series of brains and finer resolution studies to localize expression of miR-137 at cellular resolution.

To our knowledge, this is the first study to show evidence from postmortem brain that expression of miR-137 is subject to genetic regulation. We have a trend for association of miR-137 expression with the expression of TCF4, another SZ candidate gene. Taken together these data support a potential role of the miR-137 risk allele to be associated with alterations in downstream targets in brain, even though the exact timing and consequences requires further studies.

Contributors

IG designed and carried out the laboratory experiments, analyzed the data, interpreted the results and wrote the paper. AS, BR, and LM carried out the brain dissection. FT, TGMvE, and SGP contributed in the study design and in the discussion of the results. RMM, JDB, AFS, SJW, HA and WEB were involved in the conception of the study and revising the manuscript. FM assisted in the analysis and interpretation of data, and in revising the manuscript. MPV participated in the conception of the study, interpretation of the results, in writing the manuscript, and supervised the entire study.

All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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