A VNTR Regulates miR-137 Expression Through Novel Alternative Splicing and Contributes to Risk for Schizophrenia

Ashley Pacheco1, Ralph Berger1, Robert Freedman6•1 & Amanda J. Law2,2,3

The MIR137HG gene encoding microRNA-137 (miR-137) is genome-wide associated with schizophrenia (SZ), however, the underlying molecular mechanisms remain unknown. Through cloning and sequencing of individual transcripts from fetal and adult human brain tissues we describe novel pri-miR-137 splice variants which exclude the mature miR-137 sequence termed ‘del-miR-137’ that would function to down-regulate miR-137 expression. Sequencing results demonstrate a significant positive association between del-miR-137 transcripts and the length of a proximal variable number tandem repeat (VNTR) element. Additionally, a significantly higher proportion of sequenced transcripts from fetal brain were del-miR-137 transcripts indicating neurodevelopmental splicing regulation. In-silico results predict an independent regulatory function for del-miR-137 transcripts through competitive endogenous RNA function. A case-control haplotype analysis (n = 998) in SZ implicates short VNTR length in risk, with longer lengths imparting a protective effect. Rare high risk haplotypes were also observed indicating multiple risk variants within the region. A second haplotype analysis was performed to evaluate recombination effects excluding the VNTR and results indicate that recombination of the region was found to independently contribute to risk. Evaluation of the evolutionary conservation of the VNTR reveals a human lineage specific expansion. These findings shed further light on the risk architecture of the miR-137 region and provide a novel regulatory mechanism through VNTR length and alternative MIR137HG transcripts which contribute to risk for SZ.

Schizophrenia (SZ) is a neurodevelopmental disorder with complex genetic etiology. To date, 145 genetic loci have been associated through genome wide association studies (GWAS) with adult onset schizophrenia (AOS)1–4. The microRNA-137 (miR-137) gene (MIR137HG) is a top association in these studies with four regional SNPs (rs1625579, rs1198588, rs2660304, rs1702294) reaching genome wide significance. The morphological and functional complexity in the brain depends on a highly coordinated gene expression program that includes small non-coding RNAs such as microRNAs (miRs) which are critical for neurodevelopment5. MicroRNAs predominantly function to down-regulate the expression of target genes by binding to mRNA and either targeting transcripts for degradation or preventing translation. Convergent evidence from genetic, phenotypic, and functional studies support the association of miR-137 with SZ, with miR-137 gain-of-function implicated in risk. Notably, there is a significant overlap between miR-137 target genes and genes associated with SZ2,3,6,7. MIR137HG risk genotypes are associated with phenotypic variability in AOS, such as age of onset, neuroanatomical structure, and function and negative symptoms of cognitive subtypes8–11. Aberrant neurodevelopment in-utero is thought to contribute to SZ, long before the onset of clinical symptoms. This is congruent with miR-137’s association to risk and biological role in neurogenesis and early neural development, with expression of miR-137 being tightly balanced during these critical windows7,12. miR-137 acts as a gene network hub during synaptic development, functioning upstream in many critical neurodevelopmental pathways that are independently associated with SZ such as Nrg/ErbB, PI3K-Akt-mTOR, and MAPK/ERK signaling13–15. Additionally, miR-137 regulates adult neurogenesis, synaptic plasticity, and glutamatergic signaling, all processes thought to contribute to the pathobiology.

1University of Colorado, School of Medicine, Department of Psychiatry, Aurora, CO, 80045, USA. 2University of Colorado, School of Medicine, Department of Medicine, Aurora, CO, 80045, USA. 3University of Colorado, School of Medicine, Department of Cell and Developmental Biology, Aurora, CO, 80045, USA. Correspondence and requests for materials should be addressed to A.J.L. (email: amanda.law@ucdenver.edu)
of SZ. Despite these numerous associations, specific molecular mechanisms underlying miR-137’s genetic association to SZ are unclear.

A single miRNA can regulate expression of thousands of target genes, and indeed miR-137 has over 1,300 predicted targets. Therefore, even subtle changes in miRNA expression and function may have far reaching consequences. miR-137 is processed canonically through several stages and expression of miRs is largely dependent on processing efficiency. Initially the primary miR (pri-miR) is transcribed and capped and tailed. The pre-miR sequence forms a stem-loop which is cleaved by the microprocessor comprised of DROSHA and its cofactor DGCR8. The pre-miR transcript is then exported out of the nucleus and cleaved a second time to become the mature miR-137 strand. Determining how common genetic variants affect transcription and processing of miR-137 is a critical step in understanding molecular mechanisms contributing to the etiology of SZ. One variant found within MIR137HG which is proposed to alter miR-137 function, is a variable number tandem repeat (VNTR) element located within the pri-miR-137 transcript six bases upstream of the pre-miR-137 sequence. The VNTR is a repetitive sequence of 15 bases with high GC content. Three repeats is the shortest known length and the common allele. In-vitro studies show that mature miR-137 expression is affected by the length of the VNTR where greater lengths are associated with lower expression. Variations in VNTR length have also been shown to affect Stroop test performance in healthy controls. To date, the relationship between VNTR length and transcription variant of pri-miR-137 has not been examined.

The present study investigates whether the MIR137HG VNTR is associated with pri-miR-137 transcriptional variation during human brain development and contributes to the risk architecture of SZ through case-control haplotype analysis. The main objectives were (i) to characterize the transcription of MIR137HG at both early and late developmental time points through cloning and sequencing of human brain RNA, (ii) to evaluate VNTR length associations within transcript sequences, and (iii) to conduct haplotype analyses to evaluate whether the VNTR contributes to haplotype association with diagnosis. Our control cohort consisted of 307 healthy controls, 602 AOS patients, and an additional 89 childhood onset schizophrenia (COS) patients. COS is a rare form of SZ defined by the onset of psychotic symptoms before the age of 13. Previous work has determined COS to be a more genetically severe form of SZ, associated with a higher rate of copy number variants in genes associated with pathways of neuronal development and regulation. Evaluating genetic associations in multiple populations including COS is important for understanding the pathobiology of AOS and etiological differences between AOS and COS phenotypes. We predicted that GWAS SNP associations with AOS may also contribute to risk for COS, and therefore replicated SNP analysis in our COS cohort. Using a combination of molecular and bioinformatic approaches to evaluate MIR137HG transcription, we describe haplotype associations with SZ diagnosis, novel splice variants associated with VNTR length, and present new regulatory mechanisms for miR-137 which may have contributed to the evolution of increased human cognitive abilities as well as risk for complex neurodevelopmental disease.

Results
Transcript characterization. Transcription of pri-miR-137 was characterized using custom PCR primers in cDNA generated from pooled samples of human fetal and adult total brain RNA. Gel electrophoresis of PCR products exhibited a consistent multiple banding pattern for all reactions containing exon 3 (exon 1-5, exon 2-5, exon 3-5) with smaller product bands observed in addition to bands of expected size (Fig. 1a, Supplementary Fig. S1). For these reactions, amplicon base sizes ranged from approximately 350 bases less than to expected sizes (E1-5:2034, E2-5:1797, E3-5:1694). Single bands were seen for exon 4-5 and exon 5-5 amplifications (base size: E4-5:1307, E5-5:838). The observed multiple banding pattern indicates alternative splicing of pri-miR-137 transcripts within exon 3. When comparing the same primer reactions (and equal concentrations of cDNA) between fetal and adult tissues, we noted that PCR bands of expected size were brighter relative to shorter bands within the same primer reactions (and equal concentrations of cDNA) between fetal and adult groups was significantly different with a higher proportion of transcripts containing minor (＞3R) VNTR lengths in the fetal group (FET p = 5.919e-06) (Table 1). Differences in length of the VNTR between groups may reflect random population sampling differences between age groups.

Statistical transcript analyses. VNTR length frequency between age groups. The VNTR allele frequency between fetal and adult groups was significantly different with a higher proportion of transcripts containing minor (＞3R) VNTR lengths in the fetal group (FET p = 5.919e-06) (Table 1). Differences in length of the VNTR between groups may reflect random population sampling differences between age groups.

Frequency of spliced transcripts between age groups. Next, the frequency of spliced transcripts was evaluated. All transcripts that spanned the splice sites (fetal 47/56, adult 73/78) were included in the frequency analysis. The
The proportion of spliced transcripts was significantly higher (FET p = 2.145e-06) in the fetal brain group (70%) compared to the adult group (26%) (Table 1, Fig. 3b). We propose that this finding is in part due to the VNTR length association with splicing frequency (Table 1) in addition to developmental regulation, as discussed subsequently.

### VNTR Length Correlation with del-miR-137 Splicing

Direct sequencing of cloned transcripts from fetal and adult total brain cDNA allowed us to evaluate VNTR length proportions between developmental groups and VNTR length associations with alternative splicing within and at an individual transcript level. All transcripts spanning both the VNTR and the splice sites were included in analyses of VNTR length versus alternative splicing (adult n = 73, fetal n = 47, combined n = 120). Importantly, we found significant positive associations between VNTR length and proportions of spliced transcripts, in both the adult and fetal brain. When transcripts were grouped by VNTR allele (3R, >3R), for both age categories as well as combined total transcripts, a significantly higher proportion of transcripts containing VNTR lengths >3R were also del-miR-137 transcripts (FET p = 0.029 fetal, p = 3.093e-05 adult, p = 7.098e-09 combined) (Fig. 3a). These results demonstrate novel correlations between VNTR length and alternative splicing of the pri-miR-137.

### Splicing Frequency Comparisons Between 3R Groups Across Age

All transcripts containing the VNTR were included in VNTR allele frequency analyses. While it is notable that the VNTR allele frequency between fetal and adult groups was significantly different, between group analysis confirmed that the major VNTR allele (3R) in the fetal brain was associated with higher levels of del-miR-137 transcripts compared to the 3R in the adult group (FET p = 6.790e-04; Fig. 3a). This finding reflects possible developmental differences in epigenetic regulation of the region, independent of VNTR length, since VNTR genotype is consistent between groups. This observation is consistent with the previous finding of significantly higher del-miR-137 transcripts in fetal brain.

| Variant | Adult Brain (n = 73) | Fetal Brain (n = 47) | FET p |
|---------|----------------------|----------------------|-------|
| SS'     | 18                   | 1                    |       |
| SS''    | 1                    | 1                    |       |
| Total SS| 19                   | 3                    | 2.15e-06 |
| VNTR Length (n = 73) | (n = 56) |       |
| 3       | 1                    | 1                    |       |
| 4       | 0                    | 0                    | 2.36e-01 |
| 5       | 0                    | 0                    | 4.36e-01 |
| 6       | 0                    | 0                    | 4.36e-01 |
| 7       | 5                    | 6                    | 4.36e-01 |
| 9       | 1                    | 1                    | 4.36e-01 |
| Total >3| 7                    | 9                    | 5.91e-06 |

Table 1. Cloning and Sequencing Transcript Data. SS' - primary splice site, SS'' - secondary splice site.

---

**Figure 1.** Pri-miR-137 PCR Exhibits Multiple Banding Pattern. (a) PCR results are shown for pri-miR-137 custom primer pairs in adult and fetal total brain cDNA. Exon primer pairs are labeled along the top of each gel lane (i.e. E1-5 is primer pair exon 1 to exon 5). Ladders and corresponding base sizes are shown on either side. Results for both adult and fetal total brain tissues depict correct product lengths for all primer pairs (E1-5:2034, E2-5:1797, E3-5:1694, E4-5:1307, E5-5:838). A consistent multiple banding patterns can be seen for all reactions containing exon 3, indicating alternative splicing within exon 3. (b) A magnified view of PCR results for primer pair E3-5 demonstrates potential differential transcript expression between time points with brighter bands of expected size relative to shorter bands in the adult tissue and equal brightness between band sizes in the fetal tissue. (a) Images were cropped from separate gels. (b) Images cropped from the same gel. Uncropped gel images are presented in Supplemental Fig. S1.
compared to adult (Fig. 3b). All findings remain significant after controlling for multiple comparisons using the Benjamini-Hochberg procedure.

Taken together, these results implicate VNTR length in conjunction with developmentally regulated epigenetic factors as part of a novel miR-137 regulatory mechanism mediated through pri-miR-137 alternative splicing.

**CpG island prediction.** The VNTR sequence has high GC content and in order to evaluate contributions of VNTR length to regional GC content we analyzed putative CpG islands (CGIs) using EMBOSS. Results show two putative CGIs within the MIR137HG genome region one smaller 247 base CGI spanning HG19 chr1:98456956 – 98457203 and a larger 647 base CGI spanning HG19 chr1:98457215 – 98457862 (Supplementary Fig. S2). These regions overlap a large portion of pri-miR-137 exon 3, including the VNTR, pre- and mature miR-137 sequences and span into intron 3. Because the VNTR is within the CGI, increased VNTR lengths increase length of the CGI. These results indicate that the VNTR may impart regulatory effects on pri-miR-137 splicing through altered regional GC content.

**Competitive endogenous RNA in silico analyses.** The del-miR-137 transcript constitutes a novel long non-coding RNA (lncRNA) that may have independent regulatory function. LncRNAs can regulate miRNA
target gene networks through a competitive endogenous RNA (ceRNA) mechanism. CeRNAs function by containing microRNA response elements (MREs) that competitively bind and sequester miRNAs. To assess potential ceRNA functionality of the del-miR-137 transcripts, we first evaluated predicted MREs within the del-miR-137 sequence and generated a list of associated miRs using miRDB.org. We found predicted MREs for 56 miRs (Supplementary Table S1). Next, we evaluated potential target genes regulated by the top scoring miRs with target scores >60 (n = 31). A list of 1,591 previously validated target genes was generated from the predicted binding microRNAs. Pathway analysis and ranking of target genes determined ‘Axon guidance’ as the top associated pathway with 10 of the predicted del-miR-137 binding miRs known to target 45/266 total genes in the term (Supplementary Tables S1 and S2). Axon guidance pathway genes have been previously associated with risk for SZ. These results indicate potential ceRNA function for del-miR-137 transcripts which may serve to developmentally up-regulate axon guidance associated pathways through sequestration of associated miRs.

### Analysis of GWAS SNPs in childhood onset schizophrenia.

We found no significant association between SNPs rs1625579 (\(\chi^2 = 2.259, \text{df} = 2, p = 0.294\)), rs2660304 (\(\chi^2 = 1.195, p = 0.580\)) and COS diagnosis. SNP rs1198588 genotype was significantly associated with COS diagnosis (\(\chi^2 = 6.171, p = 0.048\)). The COS group had a higher frequency of minor (A/A) genotypes compared to controls (OR = 3.23, 95% CI 1.15–8.96). Interestingly, this is the opposite finding from GW AS of the adult SZ population where the risk allele is the major allele (T). Our individual variant analyses show no association between genotype at SNPs rs1625579 and rs2660304 and COS diagnosis.

### Case-control variant genotyping.

The VNTR was genotyped in all patients and controls to evaluate association with diagnosis. VNTR lengths ranged from 3 to 14 repeats (Supplementary Table S3). Previous findings as well as our current findings for VNTR length frequencies, particularly for longer repeats, vary between studies. We found through extensive troubleshooting that extended VNTR lengths (>9) require a high GC content PCR system in order to consistently amplify. We suspect that disparate allele frequencies between studies are due to different amplification protocols. Results of the VNTR case-control analysis failed to reach significance (p = 0.06), however the direction of effect indicates that the 3 repeat allele is found more frequently in patients (OR: 1.22), and we predict a significant effect may be seen with a larger sample size. Additionally, analysis of VNTR length alone is confounded by haplotype background, and potential additional risk variants within the region, we therefore next included the VNTR within a haplotype analysis.

### Haplotype analyses.

To fully assess the VNTR contribution to risk while controlling for genetic background in the region, haplotype analyses were performed using SNP and VNTR genotyping data. This analysis also allowed us to evaluate risk for more rare haplotypes. Results are shown in Fig. 4. The common haplotype (TT3T) frequency for each group is: 54.7% in controls, 57.3% in the combined patient sample, 57.5% in AOS and 56.5% in COS patients. The global haplotype analysis was significant for all diagnosis groups (Combined global p = 4.9e-03, AOS global p = 3.3e-03, COS global p = 2.6e-03) indicating overall association of the haplotype block with SZ. Tables and plots of haplotype risk scores for each diagnostic group are shown in Fig. 4. Haplotype risk scores indicate a significant protective effect for the (TT > 3T) group in the combined (p = 0.014) and AOS (p = 0.020) groups. In the COS group, the directionality of the protective effect of the (TT > 3T) was consistent but failed to reach significance (p = 0.098) likely due to the smaller sample size and lack of power in this group. Furthermore, significant haplotype associations to risk were also seen for three rare haplotypes. The combined and AOS groups showed significant association to the rare AT3G (Combined p = 0.009; AOS p = 0.007) and TG3T (Combined p = 0.006; AOS p = 0.001) haplotypes. The COS groups also had a significant score for the rare haplotype (AT3T p = 1.0e-5). Together, these results confirm overall association within the MIR137HG region to SZ and suggest combinatorial effects of the VNTR and the GWAS SNPs leading to both protective and rare high risk haplotype associations in patients with AOS and COS.

Results of the individual haplotype analysis allowed us to assess magnitude and direction of effect for significant haplotypes (Table 2). We used the protective haplotype (TT > 3T) as the reference to evaluate risk effects for the major haplotype. Results are consistent with the global haplotype score results. For the combined diagnosis group, individual haplotype analysis found significant effects for the common haplotype TT3T (OR: 1.32, p = 0.035), as well as for the rare haplotype AT3G (OR: 10.49, p = 0.022), and the rare haplotype group (OR: 2.36, p = 0.024). When the minimum frequency is lowered to 0.8% to allow for evaluation of the TG3T haplotype, complete separation is seen in the model with the TG3T haplotype only seen in cases and not in controls, and the rare haplotype group is no longer significant (p = 0.507). This complete separation confirms the global haplotype result that the TG3T haplotype is a rare high risk haplotype and indicates that the TG3T haplotype is driving the risk association with the rare haplotype group. An increased sample size is further needed to better assess the TG3T haplotype within the individual GLM model. Similar results were found for the AOS diagnosis group with significant risk associations for haplotypes TT3T (OR: 1.34, p = 0.034), AT3G (OR: 11.81, p = 0.016), and the rare haplotype group (OR: 2.22, p = 0.013). With the minimum haplotype frequency lowered to 0.9%, again we see complete separation with the TG3T haplotype being only found in patients, and the rare haplotype group becoming non-significant (p = 0.219). The risk association of the common TT3T haplotype relative to the TT > 3T haplotype implicates the major VNTR 3R allele as a driver of risk for the TT3T haplotype. Genotyping allowed us to conduct LD analysis of the VNTR and GWAS SNPs within our control cohort. Results indicate low linkage between the VNTR and GWAS SNPs (VNTR LD versus: rs1198588 D’ = 0.03, r = 0.02; rs2660304 D’ = 0.04, r = 0.03; rs1625579 D’ = 0.02, r = 0.01) suggesting that VNTR instability is not haplotype dependent, and that GWAS SNPs would not be tagging the VNTR. This is consistent with previous VNTR LD findings and supports a role for other regional risk variants.
It is apparent from our data that the VNTR is not the only risk variant within the region as evidenced by low LD with the GW AS SNPs, the lack of risk association with the AG3G haplotype and high risk associations found with the rare AT3G and TG3T haplotypes. The high risk of these rare haplotypes suggest that recombination of the rs2660304 SNP region may also be contributing to risk. In order to evaluate how recombination alone may contribute to risk independent of the VNTR, we conducted global and individual haplotype analyses utilizing only SNP genotypes, excluding the VNTR variant. Results of these analyses confirm that recombination of the rs2660304 region alone is a driver of risk (Global haplotype scores: Combined: TGT $p = 2.8 \times 10^{-3}$, ATG $p = 1.9 \times 10^{-3}$; AOS: TGT $p = 1.2 \times 10^{-3}$, ATG $p = 9.9 \times 10^{-3}$. Individual GLM: Combined TGT OR: 12.24 $p = 0.015$, ATG OR: 12.22 $p = 0.015$; AOS TGT OR: 14.40 $p = 9.0 \times 10^{-3}$, ATG OR: 13.27 $p = 0.012$). These results suggest that recombination of the rs2660304 region contributes to risk independently of VNTR genotype, through a presently unknown mechanism.

A different pattern of association was found for COS diagnosis. The rare haplotype AT3T was significant (haplo.glm OR: 10.28, $p = 8.0 \times 10^{-4}$), and no association was found with the common TT3T haplotype. This result is consistent with our previous data demonstrating a genotype association with the minor rs1198588 genotype (A/A) and COS diagnosis. Further functional studies will be needed to confirm risk association. As seen in Table 2, confidence intervals for rare haplotype associations are large due to low haplotype frequencies, larger sample sizes will be needed to determine more accurate effect sizes and replicability of the association findings. Overall, these results demonstrate a complex pattern of risk architecture for the MIR137HG region with shorter VNTR lengths and recombination of the rs2660304 region both independently contributing to risk for SZ diagnosis.

Human specific VNTR expansion. Previous research demonstrates a significant correlation between VNTR genotype and cognitive phenotypes. We hypothesize that expansion of the VNTR is evolutionarily advantageous for cognition, due to its potential negative regulation of miR-137. To evaluate the VNTR in the...
In the context of mammalian evolution, we used Ensembl Comparative Genomics to evaluate conservation. Sequence alignment shows that the VNTR length of 2 repeats and the surrounding region is highly conserved throughout the primate lineage (Fig. 5). However, the expansion of the VNTR to 3 repeats is human lineage specific. This result suggests that VNTR expansion may have contributed to increased human cognitive abilities and may contribute to risk for Autism Spectrum Disorder (ASD) as well as SZ.

Discussion

Through this work, we report novel miR-137 regulatory mechanisms and further characterize SZ risk architecture of the MIR137HG genome region. Transcript characterization at early and late developmental stages (fetal vs. adult) revealed a novel pri-miR-137 transcript isoform, del-miR-137. Expression of this lncRNA would down-regulate mature miR-137 expression through alternative 5' splicing within the pri-miR-137 sequence. Importantly, we found a significant positive association with del-miR-137 transcripts and VNTR length. Increased VNTR lengths were observed more frequently within del-miR-137 transcripts. Analysis of regional GC content demonstrates that the VNTR contributes to length of an overlapping CpG island, which may underlie
VNTR associations with alternative splicing. We describe common MIR137HG haplotype associations with SZ which are driven by VNTR length, in addition to more rare high risk haplotypes. These associations reveal multiple epistatic risk variants and overall complex risk architecture for the region, that may underlie the common GWAS associations to SZ.

Our present findings are consistent with previous GWAS findings where the common variants are the risk variants. Haplotype analyses revealed risk association with the common TT3T haplotype to increased risk. Critically, our findings demonstrate that the VNTR is a driver of risk only on the background of the common haplotype, as the TT > 3T haplotype imparts a protective effect. In conjunction with our finding that increased VNTR lengths are associated with del-miR-137 splice variants, we predict that the VNTR may mediate risk through altered GC content with the potential to affect DNA binding proteins such as methyl-CpG binding protein 2 (MeCP2). MeCP2 has been shown to up-regulate alternative splicing and down-regulate miRNA processing and in this context may contribute to the protective effect of longer VNTR lengths. Functional studies to test this hypothesis are currently underway.

Additionally, we describe a putative novel ceRNA function for the del-miR-137 transcript which may alter axon guidance and other neurodevelopmental pathways and provide additional protective effects for alternatively spliced transcripts independent of reduced miR-137 expression. In general our results suggest that shorter VNTR lengths may contribute to risk for observed miR-137 over-expression in SZ patients and longer VNTR lengths may impart a protective effect through decreased miR-137 expression. Interestingly, rare high risk haplotypes with recombination of the rs2660304 region were identified. Specific underlying risk mechanisms are unknown, however we predict that cis-acting repressive elements for both the major (TTT) and minor (AGG) haplotypes would be disrupted by recombination. This would result in increased miR-137 expression for the recombinated (TTT and ATG) haplotypes, which would contribute to gain-of-function risk for SZ. Replication of our findings in a larger population cohort and further functional analyses is needed to validate these high risk associations and elucidate risk mechanisms.

The human lineage specific expansion of the VNTR supports a potential role for this variant in the evolution of increased cognitive abilities in humans. Longer VNTR repeats would result in a competitive cognitive advantage associated with suppression of miR-137 expression. This is supported by GWAS associations with intelligence and cognitive performance within the MIR137HG locus. Additionally, VNTR length may contribute to risk for other neurodevelopmental disorders. While SZ and ASD share a subset of phenotypes and risk variants, there are several known pathological variants and pathways in which the direction of risk for SZ is the opposite of risk direction for ASD, also known as the diametric hypothesis. The MIR137HG locus follows this diametric pattern of association. Findings from GWAS have identified minor allele SNPs within the MIR137HG locus to be associated with ASD, which is the opposite finding for GWAS SZ SNPs which are all major alleles. Conditional knockout of miR-137 in mice lead to social deficits and unusual repetitive behavior, phenotypes that are associated with ASD. Individuals with microdeletion of MIR137HG had a syndrome presenting with intellectual disability, ASD, and obesity. We predict that longer VNTR lengths may confer risk for ASD as miR-137 loss-of-function results in ASD like phenotypes. These findings support an evolutionary role for VNTR expansion in the development of the human brain.

This work contributes to our understanding of the molecular mechanisms underlying the diagnostic associations with common MIR137HG genetic variants and SZ. In totality, our findings highlight the complex neurodevelopmental regulation of miR-137 and contribute to our understanding of its role in SZ etiology.

Methods

PCR. Complimentary DNA (cDNA) libraries were constructed using standard protocols for the Oligo(dT) primed SuperScript®III First-Strand Synthesis System from commercial human brain tissues (Human Fetal Brain Total RNA from Clontech Cat# 636526 and adult FirstChoice Human Reference Brain Cat# AM6051). Fetal Brain RNA was derived from normal fetal brains pooled from 59 spontaneously aborted male/female Caucasian fetuses, ages: 20–33 weeks. Adult Human Brain RNA was pooled from 21 adult Caucasian individuals. PCR was conducted using standard primer pairs and standard protocols for Platinum™ Taq DNA Polymerase High Fidelity (ThermoFisher Cat# 11304029). The pri-miR-137 (UCSC ID uc001drx.2) transcript contains 5 exons and 4 introns. Primer pairs were designed with a 5′ tailing of PCR products to improve cloning efficiency. Cells were plated on either 50 ug/ml kanamycin or 100 ug/ml ampicillin agar plates and blue and white screening was used to select vector containing colonies. Colonies were transferred to a 100 mg/ml ampicillin LB broth starter culture overnight and then 1:500 dilutions were cultured for an additional 13–14 hours. Plasmid DNA was isolated using standard protocols for the Qiagen®Spin Miniprep kit.

Cloning. Cloning of total PCR products was conducted using the TOPO®TA®Cloning Kit with pCR™2.1-TOPO®vector and One Shot® Chemically competent cells. Platinum Taq DNA Polymerase was used for 3′ A-tailing of PCR products to improve cloning efficiency. Cells were plated on either 50 ug/ml kanamycin or 100 ug/ml ampicillin agar plates and blue and white screening was used to select vector containing colonies. Colonies were transferred to a 100 mg/ml ampicillin LB broth starter culture overnight and then 1:500 dilutions were cultured for an additional 13–14 hours. Plasmid DNA was isolated using standard protocols for the QIAprep®Spin Miniprep kit.

Sequencing. Sequencing of plasmid DNA was conducted through GENEWIZ® using M13 forward and reverse primers. Sequences were aligned to the reference genome using NCBI BLAST® and CodonCode Aligner software (www.codoncode.com/aligner/index.htm). Novel sequences were deposited to GenBank and assigned the following accession numbers: MK631885, MK631886, MK631887.

Statistical analyses. Sequenced transcript analyses. Associations between spliced transcripts, developmental time points (fetal, adult) and VNTR length were evaluated using Fisher’s exact test (FET), p-values of
<0.05 were considered as statistically significant. Transcripts were grouped by age (Adult Brain,Fetal Brain) as well as by VNTR length (3, >3 repeats). Proportions of del-miR-137 transcripts were compared between age groups and between total transcripts grouped by VNTR length. Proportion of del-miR-137 transcripts was compared between VNTR length groups, within age groups (i.e. adult 3R vs. adult >3R). Finally, proportions of del-miR-137 transcripts was compared between age groups keeping VNTR length consistent (i.e. 3R Adult vs. 3R Fetal). Multiple comparisons were corrected for using the Benjamini-Hochberg procedure.

**Case-control genotype analyses.** VNTR length was nominally grouped as either 3R (major allele) or >3R (minor allele) based on genetic and biological findings regarding the VNTR21,22 and evaluated using a two-tailed Pearson's Chi-squared test between combined AOS and COS patients and healthy controls.

For COS genotype associations two-tailed Pearson's Chi-squared test based on a permutation distribution was used. Fisher's exact test (FET) with mid-p correction was used to calculate odds ratios (OR) and confidence intervals (CIs). Multiple testing correction was not performed as we considered this a replication analysis of previous associations in SZ; p-values of <0.05 were considered as statistically significant. These results will be used as a point for initial inquiry and not to make determinative conclusions.

**CpG island prediction.** EMBOSS CpGplot was used to evaluate CpG content and putative CpG islands (CGIs) within the MIR137HG genome region. Default settings were used: window size = 100, minimum length of an island = 200 bases, minimum average observed/expected ratio of C plus G to CpG = 0.6, minimum average percentage of G plus C = 50.

**Subjects.** All study procedures were approved by the Colorado Multiple Institutional Review Board, University of Colorado and samples collected in accordance with the Declaration of Helsinki principles with the appropriate consent. All clinical subjects were drawn from individuals participating in the Department of Psychiatry ‘Genetic Research in Schizophrenia’, part of a larger original study of the Molecular Genetics of Schizophrenia Consortium, and as described previously48. A consensus diagnosis of SZ based on DSM-IIIR orDSM-IV criteria was made following a systematic examination of multiple sources of available information obtained from relatives, medical records, clinicians, and direct assessment using one or more diagnostic interviews including the Diagnostic Interview for Genetic Studies (DIGS), the Structured Clinical Interview for Axis I DSM Disorders (SCID). Control subjects were evaluated using the SCID-Non-patient Edition. All subjects provided informed consent to partake in the study. COS subjects were recruited through the Colorado Childhood-onset Schizophrenia Research Program at the University of Colorado School of Medicine49,50. Diagnosis of COS was made after the Schedule for Affective Disorders and Schizophrenia for Schoolage Children - Present and Lifetime Version (KSADS- PL) was administered to one parent and the child. Assent by the child, and informed consent was obtained from one or both parents. Criteria for diagnosis were identical to those utilized in adult populations and as described previously49,50. All subjects in this study were unrelated to each other. Where postmortem brain DNA samples were used these were obtained from the Schizophrenia Research Center Brain Bank at the University of Colorado, School of Medicine, all of which were donated through the Colorado Anatomical Gift Act and donation approved by the donor family. Ethical approval was obtained for postmortem sample collection and use from the Colorado Multiple Institutional Review Board, University of Colorado and classified as exempt ‘not human tissue research-autopsy’. The Brain Bank contains tissue collected postmortem from subjects with a diagnosis of SZ as well as from unaffected individuals51. DNA was isolated using standard methods, from unrelated Caucasian individuals (AOS (n = 602) COS subjects (n = 89) and control (n = 307); See Table 3 for demographics). Subjects were included for analyses only if all variants were successfully genotyped.

### Table 3. Subject Demographics. COS- childhood onset schizophrenia; AOS- adult onset schizophrenia. *Age at collection is given for samples from blood draws, age at death is given for brain samples, age at first visit to clinic typically coinciding with age of diagnosis is given for COS samples.

| Subjects | N   | Mean Age ± SE | No. (%) male |
|----------|-----|---------------|--------------|
| Controls | 367 | 51.0 ± 0.85   | 161 (52.4%)  |
| COS      | 89  | 51.1 ± 0.28   | 61 (68.5%)   |
| AOS      | 602 | 42.2 ± 0.53   | 421 (69.9%)  |

**Genotyping.** Three GWAS significant SNPs (rs1625579, rs1198588, rs2660304) were genotyped for all individuals. All SNPs were in Hardy-Weinberg equilibrium in controls (rs1625579: χ² = 0.275, p = 0.600; rs1198588: χ² = 0.039, p = 0.844; rs2660304: χ² = 0.275, p = 0.600). Genotyping was performed in duplicate for each sample using TaqMan Genotyping Assays and qPCR on a 7900HT Fast Real-Time PCR System. Samples were excluded if genotype calls were undetermined or if discrepant calls were made between duplicates after two rounds of genotyping. Multiple testing correction was not performed as we considered this a replicative analysis of previous associations in SZ; p-values of <0.05 were considered as statistically significant.

An optimized PCR protocol for the VNTR was used to genotype all individuals with custom VNTR primers (Forward: 5’ GCTCAGCGGAGCAAGAAGT; Reverse: 5’ GTACAGAAAGAGTCAGAGGACC) using the GC-RICH PCR System mfr. Roche. PCR products were run on a 3% agarose gel to resolve the 15 bp repeat size differences. After a sufficient number of samples were run, a band size ladder was generated with VNTR lengths ranging from 3–12 repeats. Ladder results were confirmed through DNA Fragment analysis (GENEWIZ) with a fluorescent FAM labeled primer (FAM labeled: 5’ TCACAGGAGAGGCAGAGGACC). Manual calls were

---

**CpG island prediction.** EMBOSS CpGplot was used to evaluate CpG content and putative CpG islands (CGIs) within the MIR137HG genome region. Default settings were used: window size = 100, minimum length of an island = 200 bases, minimum average observed/expected ratio of C plus G to CpG = 0.6, minimum average percentage of G plus C = 50.

**Subjects.** All study procedures were approved by the Colorado Multiple Institutional Review Board, University of Colorado and samples collected in accordance with the Declaration of Helsinki principles with the appropriate consent. All clinical subjects were drawn from individuals participating in the Department of Psychiatry ‘Genetic Research in Schizophrenia’, part of a larger original study of the Molecular Genetics of Schizophrenia Consortium, and as described previously48. A consensus diagnosis of SZ based on DSM-IIIR orDSM-IV criteria was made following a systematic examination of multiple sources of available information obtained from relatives, medical records, clinicians, and direct assessment using one or more diagnostic interviews including the Diagnostic Interview for Genetic Studies (DIGS), the Structured Clinical Interview for Axis I DSM Disorders (SCID). Control subjects were evaluated using the SCID-Non-patient Edition. All subjects provided informed consent to partake in the study. COS subjects were recruited through the Colorado Childhood-onset Schizophrenia Research Program at the University of Colorado School of Medicine49,50. Diagnosis of COS was made after the Schedule for Affective Disorders and Schizophrenia for Schoolage Children - Present and Lifetime Version (KSADS- PL) was administered to one parent and the child. Assent by the child, and informed consent was obtained from one or both parents. Criteria for diagnosis were identical to those utilized in adult populations and as described previously49,50. All subjects in this study were unrelated to each other. Where postmortem brain DNA samples were used these were obtained from the Schizophrenia Research Center Brain Bank at the University of Colorado, School of Medicine, all of which were donated through the Colorado Anatomical Gift Act and donation approved by the donor family. Ethical approval was obtained for postmortem sample collection and use from the Colorado Multiple Institutional Review Board, University of Colorado and classified as exempt ‘not human tissue research-autopsy’. The Brain Bank contains tissue collected postmortem from subjects with a diagnosis of SZ as well as from unaffected individuals51. DNA was isolated using standard methods, from unrelated Caucasian individuals (AOS (n = 602) COS subjects (n = 89) and control (n = 307); See Table 3 for demographics). Subjects were included for analyses only if all variants were successfully genotyped.

### Table 3. Subject Demographics. COS- childhood onset schizophrenia; AOS- adult onset schizophrenia. *Age at collection is given for samples from blood draws, age at death is given for brain samples, age at first visit to clinic typically coinciding with age of diagnosis is given for COS samples.

| Subjects | N   | Mean Age ± SE | No. (%) male |
|----------|-----|---------------|--------------|
| Controls | 367 | 51.0 ± 0.85   | 161 (52.4%)  |
| COS      | 89  | 51.1 ± 0.28   | 61 (68.5%)   |
| AOS      | 602 | 42.2 ± 0.53   | 421 (69.9%)  |
100% consistent with fragment analysis results. This ladder was used for comparison to genotype remaining individuals manually. Linkage disequilibrium (LD) between the VNTR and GWAS SNPs was calculated in the control cohort using the R package ‘genetics’ LD function.

**Haplotype analyses methods.** Haplotype analysis was conducted using R haplo.score and haplo.glm functions. Input data included unphased genotypes for all GWAS SNPs (rs1625579, rs1198588, rs25660304) and the VNTR for each individual. Global score tests of association use an additive GLM to regress diagnosis on haplotype pairs for an individual. Correction for multiple testing was performed by applying the simulate = TRUE parameter in haplo.score which provides simulated p-values for each haplotype. Haplotypes were excluded with frequencies <0.8% for the combined diagnosis group, and <1% for AOS and COS groups. The magnitude and direction of individual haplotype effects is determined using a Wald type test relative to a common reference haplotype. Initially, rare haplotypes (<0.8%) were grouped and included in the model. As complete separation was seen for the TG3T haplotype, minimum haplotype frequency was raised to 0.9% to include the TG3T haplotype with the rare group and better fit the model. In order to evaluate effects the common haplotype, the second most frequent haplotype was used as the reference. An additional haplotype analysis was conducted using only GWAS SNPs to evaluate VNTR independent associations.

**VNTR conservation.** Ensembl (release 95) Comparative Genomics was used to evaluate the conservation of the VNTR in the 75 eutherian alignments set. The evaluated sequence included GRCh38.p12 Chromosome 1: 98,046,166-98,046,254.

**ceRNA pathway.** The miRDB.org website was used to generate a list of predicted miRs that bind to the del-miR-137 transcripts. The miRDB tool uses a custom target prediction algorithm, MirTarget v4.0, which combines CLIP binding data and miRNA overexpression data to predict microRNA binding sites. The algorithm assigns target prediction scores between 50–100. Per miRDB recommendations, only miRNAs with a target score >60 were used for further analysis. This list of miRNAs was then input into the miRSystem to determine potential target genes and conduct pathway ranking analysis for target gene pathways. MiRSystem utilizes 7 algorithms predicting miRNA targets and two experimentally validated databases of miRNA target genes. Pathway analysis results are based solely on experimentally validated miR/target gene pairs.

**Data Availability**
The cDNA sequences generated and analyzed during the current study are available in the Genbank repository, accession codes: MK631885, MK631886 and MK631887. Other datasets generated and analyzed during the current study are available from the corresponding author on request.

**References**

1. Ripke, S. et al. Genome-wide association study identifies five new schizophrenia loci. *Nature genetics* 43, 969–976 (2011).
2. Ripke, S. et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet* 45, 1150–1159, https://doi.org/10.1038/ng.2742 (2013).
3. Schizophrenia Working Group of the Psychiatric Genomics, C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511, 421–427, https://doi.org/10.1038/nature13595 (2014).
4. Pardinas, A. F. et al. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nat Genet* 50, 381–389, https://doi.org/10.1038/s41588-018-0059-2 (2018).
5. Stiles, J. & Fernig, T. L. The basics of brain development. *Neuropsychol Rev* 20, 327–348, https://doi.org/10.1007/s11065-010-9148-4 (2010).
6. Kwon, E., Wang, W. & Tsai, L.-H. Validation of schizophrenia-associated genes csmd1, c10orf26, cacna1c and tcf4 as mir-137 targets. *Mol Psychiatry* 18, 11–12, https://doi.org/10.1038/mp.2011.179 (2013).
7. Wright, C., Turner, J. A., Calhoun, V. D. & Perrone-Bizzozero, N. Potential impact of mir-137 and its targets in schizophrenia. *Front Genet* 4, 58, https://doi.org/10.3389/fgene.2013.00058 (2013).
8. Kuswanto, C. N. et al. 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* 168, 317–326, https://doi.org/10.1002/ajmg.b.32314 (2015).
9. Lett, T. A. et al. The genome-wide supported microrna-137 variant predicts phenotypic heterogeneity within schizophrenia. *Mol Psychiatry* 18, 443–450, https://doi.org/10.1038/mp.2013.17 (2013).
10. Whalley, H. C. et al. Impact of a microrna mir137 susceptibility variant on brain function in people at high genetic risk of schizophrenia or bipolar disorder. *Neuropsychopharmacology* 37, 2720–2729, https://doi.org/10.1038/npp.2012.137 (2012).
11. Green, M. J. et al. Genome-wide supported variant mir-137 and severe negative symptoms predict membership of an impaired cognitive subtype of schizophrenia. *Mol Psychiatry* 18, 774–780, https://doi.org/10.1038/mp.2012.84 (2013).
12. Colantuoni, C. et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature* 478, 519–523, https://doi.org/10.1038/nature10524 (2011).
13. Loohuis, N. F. O. et al. The schizophrenia risk gene mir-137 acts as a hippocampal gene network node orchestrating the expression of genes relevant to nervous system development and function. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 73, 109–118, https://doi.org/10.1016/j.pnpbp.2016.02.009 (2017).
14. Guella, I. et al. Analysis of mir-137 expression and rs1625579 in dorsolateral prefrontal cortex. *Journal of Psychiatric Research* 47, 1215–1221, https://doi.org/10.1016/j.jpsychires.2013.05.021 (2015).
15. Thomas, K. T. et al. Inhibition of the schizophrenia-associated microRNA mir-137 disrupts nr1g1-alpha neurodevelopmental signal transduction. *Cell Reports* 20, 1–12, https://doi.org/10.1016/j.celrep.2017.06.038 (2017).
16. Hill, M. J. et al. Transcriptional consequences of schizophrenia candidate mir-137 manipulation in human neural progenitor cells. *Schizophr Res* 153, 225–230, https://doi.org/10.1016/j.schres.2014.01.034 (2014).
17. Siegert, S. et al. The schizophrenia risk gene product mir-137 alters presynaptic plasticity. *Nature Neuroscience* 18, 1008 EP– (2015).
18. Loohuis, N. F. O. et al. MicroRNA-137 controls ampa receptor-mediated transmission and mglur-dependent ltd. *Cell Reports* 11, 1876–1884, https://doi.org/10.1016/j.celrep.2015.05.040 (2015).
19. Agarwal, V., Bell, G. W., Nam, J.-W. & Bartel, D. P. Predicting effective microRNA target sites in mammalian miRNAs. *elife* 4, https://doi.org/10.7554/elif.05005 (2015).
20. Creugn, A., Fender, A. & Pfeffer, S. Regulation of primary microrna processing. FEBS Letters 592, 1980–1996, https://doi.org/10.1002/1873-3468.13067 (2018).
21. Warburton, A., Breen, G., Rujescu, D., Bubb, V. J. & Quinn, J. P. Characterization of a rest-regulated internal promoter in the schizophrenia genome-wide associated gene mir137. Schizophr Bull 41, 698–707, https://doi.org/10.1093/schbul/sbu117 (2015).
22. González-Giraldo, Y., González-Reyes, R. E. & Forero, D. A. A functional variant in mir137, a candidate gene for schizophrenia, affects strop test performance in young adults. Psychiatry Research 236, 202–205, https://doi.org/10.1016/j.psychres.2016.01.006 (2016).
23. Ahn, K. et al. High rate of disease-related copy number variations in childhood onset schizophrenia. Mol Psychiatry 19, 568–572, https://doi.org/10.1038/mp.2013.59 (2014).
24. Salmena, L., Poliseno, L., Tay, Y., Kats, L. & Pandolfo, P. P. A cerna hypothesis: the rosetta stone of a hidden rna language? Cell 146, 353–358, https://doi.org/10.1016/j.cell.2011.07.014 (2011).
25. Wong, N. & Wang, X. mirdb: an online resource for microrna target prediction and functional annotations. Nucleic Acids Res 43, D146–52, https://doi.org/10.1093/nar/gku1104 (2015).
26. Lu, T.-P. et al. mirsystem: an integrated system for characterizing enriched functions and pathways of microrna targets. PLoS One 7, e42390, https://doi.org/10.1371/journal.pone.0042390 (2012).
27. Wang, Z. et al. Axon guidance pathway genes are associated with schizophrenia risk, Experimental and Therapeutic Medicine. https://doi.org/10.3892/etm.2018.6781 (2018).
28. Mamdani, M. et al. Differential regulation of schizophrenia-associated microrna gene function by variable number tandem repeats (vnt) polymorphism. Schizophr Res 151, 284–286, https://doi.org/10.1016/j.schres.2013.10.024 (2013).
29. Stratil, M. et al. Mir137 variants identified in psychiatric patients affect synaptogenesis and neuronal transmission gene sets. Molecular Psychiatry 20, 472–473 (2014).
30. Sikela, J. M. & Seazles Quick, V. B. Genomic trade-offs are autism and schizophrenia the steep price of the human brain? Hum Genet 137, 1–13, https://doi.org/10.1007/s00439-017-1865-9 (2018).
31. Beveridge, N. J., Gardiner, E., Carroll, A. P., Tooney, P. A. & Cairns, M. J. Schizophrenia is associated with an increase in cortical microrna biogenesis. Molecular Psychiatry 15, 1176–EP (2009).
32. Santarelli, D. M., Beveridge, N. J., Tooney, P. A. & Cairns, M. J. Upregulation of dicer and microrna expression in the dorsolateral prefrontal cortex brodmann area 46 in schizophrenia. Biological Psychiatry 70, 180–187, https://doi.org/10.1016/j.biopych.2010.09.030, Postmortem Studies of Psychosis: Status, Opportunities, and Challenges (2011).
33. He, E. et al. Mir137 schizophrenia-associated locus controls synaptic function by regulating synaptogenesis, synapse maturation and synaptic transmission. Human Molecular Genetics 27, 1879–1891, https://doi.org/10.1093/hmg/ddy089 (2018).
34. Savage, J. E. et al. Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. Nat Genet 50, 912–919, https://doi.org/10.1038/ng.3858-018-0152-6 (2018).
35. Davies, G. et al. Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. Nat Commun 9, 2098, https://doi.org/10.1038/s41467-018-04362-x (2018).
36. Rietveld, C. A. et al. Common genetic variants associated with cognitive performance identified using the proxy-phenotype method. Proc Natl Acad Sci USA 111, 13790–13794, https://doi.org/10.1073/pnas.1404621111 (2014).
37. Lee, J. J. et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. Nat Genet 50, 1112–1121, https://doi.org/10.1038/s41588-018-0147-3 (2018).
38. Crespi, B. & Badcock, C. Psychosis and autism as diametrical disorders of the social brain. Behav Brain Sci 31, 241–261, https://doi.org/10.1017/S0140525X08004214 (2008).
39. Crespi, B., Stead, P. & Elliot, M. Evolution in health and medicine sackler colloquium: Comparative genomics of autism and schizophrenia. The American Journal of Human Genetics 95, 677–694, https://doi.org/10.1016/j.ajhg.2014.03.018 (2014).
40. Willemsen, M. H. et al. Chromosome 1p21.3 microdeletions comprising dpyd and mir137 are associated with intellectual disability. Journal of Medical Genetics 48, 810–818, https://doi.org/10.1136/jmedgenet-2011-100294, https://jmg.bmj.com/content/48/12/810.full.pdf (2011).
41. D'Angelo, C. S., Moller Dos Santos, M. F., Alonzo, L. G. & Koiffmann, C. P. Two new cases of 1p21.3 deletions and an unbalanced translocation t(8;12) among individuals with syndromic obesity. Mol Syndromol 6, 63–70, https://doi.org/10.1159/000371600 (2015).
42. Tucci, A., Ciaccio, C., Scuvera, G., Esposito, S. & Milani, D. Mir137 is the key gene mediator of the syndromic obesity phenotype of patients with 1p21.3 microdeletions. Mol Cytogenet 9, 80, https://doi.org/10.1186/s13039-016-0289-x (2016).
43. Johnson, M. et al. Ncbi blast: a better web interface. Nucleic Acids Res 36, W5–9, https://doi.org/10.1093/nar/gkn201 (2008).
44. Sinkus, M. L. et al. A 2-base pair deletion polymorphism in the partial duplication of the alpha7 nicotinic acetylcholine gene (chrfam7a) on chromosome 15q14 is associated with schizophrenia. Brain Research 1291, 1–11, https://doi.org/10.1016/j.brainres.2009.07.041 (2009).
45. Ross, R. G., Heinlein, S. & Tregellas, H. High rates of comorbidity are found in childhood-onset schizophrenia. Schizophrenia Research 88, 90–95, https://doi.org/10.1016/j.schres.2006.07.006 (2006).
46. Brodsky, K., Willcutt, E. G., Davalos, D. B. & Ross, R. G. Neuropsychological functioning in childhood-onset psychosis and attention-deficit/hyperactivity disorder. Journal of Child Psychology and Psychiatry 55, 811–818, https://doi.org/10.1111/jcpp.12199 (2014).
47. Sinkus, M. L., Adams, C. E., Logel, I., Freedman, R. & Leonard, S. Expression of immune genes on chromosome 6p21.3-22.1 in schizophrenia. Brain Behav Immun 32, 51–62, https://doi.org/10.1016/j.bbi.2013.01.087 (2013).
48. Duan, J. et al. A rare functional noncoding variant at the gwas-implicated mir137/mir2682 locus might confer risk to schizophrenia and bipolar disorder. The American Journal of Human Genetics 95, 744–753, https://doi.org/10.1016/j.ajhg.2014.11.001 (2014).
49. Sinnwell, J. P. & Schaid, D. Statistical Analysis of Haplotypes and Microrna Traits and Variates when Linkage Phase is Ambiguous (2018).
50. Liu, W. & Wang, X. Prediction of functional microrna targets by integrative modeling of microrna binding and target expression data. Genome Biology 20, 18, https://doi.org/10.1186/s13059-019-1629-z (2019).

Acknowledgements
This work was supported by the National Institutes of Mental Health under Award Number R01MH59565 (RF) and funds from the University of Colorado and the Dr. Nancy Gary Endowed Chair (AJL). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
Author Contributions
A.J.L. and A.P. conceived and designed all experiments. A.P. and R.B. performed cloning, sequencing, and genotyping experiments. A.P. performed statistical and bioinformatic analyses under the supervision of A.J.L., A.P. and A.J.L. participated in all data interpretation. A.J.L. and A.P. wrote the manuscript and prepared the figures. R.F. conceived and established the original human subject studies and provided samples. All authors reviewed, provided editorial advice and approved the final manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-48141-0.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019