De novo mutations involved in post-transcriptional dysregulation contribute to six neuropsychiatric disorders

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Abstract

While de novo mutations (DNMs) conferring risk in neuropsychiatric disorders have been revealed by next-generation sequencing, the role of DNMs involved in post-transcriptional regulation in pathogenesis of these disorders remains to be elucidated. By curating 42,871 DNMs from 9,772 core families with six kinds of neuropsychiatric disorders and controls, we identified 2,381 post-transcriptionally impaired DNMs (piDNMs) and prioritized 1,923 candidate casual genes in these six disorders by employing workflow RBP-Var. Our results revealed a higher prevalence of piDNM in the probands than that of controls ($P = 9.52E-17$). Moreover, we identified 214 piDNM-containing genes with shared enriched co-expression modules and intensive protein-protein interactions ($P = 7.75E-07$) in at least two of neuropsychiatric disorders. Furthermore, these cross-disorder genes carrying piDNMs could form interaction network centered on RNA binding proteins, suggesting a shared post-transcriptional etiology underlying these disorders. Our findings highlight that piDNMs contribute to the pathogenesis of neuropsychiatric disorders.
**Introduction**

In the past decades, there have been increased efforts to better understand the pathogenesis of psychiatric disorders. Next-generation sequencing, which allows genome-wide detection of rare and *de novo* mutations (DNMs), is transforming the pace of genetics of neuropsychiatric disorder by identifying protein-coding mutations that confer risk. Despite previous studies that have analyzed the biological and clinical implications of protein truncating mutations identified from next-generation sequencing,\(^1,^2\) the risk of DNMs involved in transcriptional and post-transcriptional regulation in these disorders remain obscure.

Various computational methods have been developed to predict the effects of amino acid substitutions on protein function and to classify corresponding mutations as deleterious or benign. The majority of these approaches rely on evolutionary conservation or protein structural constraints of amino acid substitutions and focus on direct changes in protein coding genes, especially from nonsynonymous mutations which directly affect the gene product\(^3\). However, genetic mutations may not only impact the protein structure or catalytic activity but may also influence transcription\(^4,^5\) via direct or indirect effects on DNA-protein binding\(^6\), histone modifications\(^7\), enhancer cis-regulation\(^8\) and gene-enhancer interactions\(^9\). Meanwhile, genetic mutations could impair post-transcriptional processes\(^10,^11\) such as interactions with RNA binding proteins (RBPs), mRNA splicing, mRNA transport, mRNA stability and mRNA translation.

Hitherto, previous studies of neuropsychiatric disorders have focused on genetic mutation in coding region\(^12\), cis-regulation\(^13-15\), epigenome\(^16\), transcriptome\(^17,^18\), and proteome\(^19,^20\), but less is pursued with regard to how these disorders interface with post-transcriptional regulation. Therefore, potential
mechanisms of post-transcriptional dysregulation related to pathology and clinical treatment of neuropsychiatric disorders remain largely uninvestigated. Our previous method named RBP-Var\textsuperscript{21} and a recent published platform called POSTAR\textsuperscript{22} represents a seminal effort to systematically annotate post-transcriptional regulatory maps and explore the putative roles of RBP-mediated SNVs in human diseases.

To explore association between extremely post-transcriptionally impaired DNM s, namely piDNMs, with psychiatric disorders, we collected whole exome and genome sequencing data from 9,772 core family and curated 42,871 \textit{de novo} mutations from six kinds of neuropsychiatric disorders, including autism spectrum disorder (ASD), epileptic encephalopathy (EE), intellectual disability (ID), schizophrenia (SCZ), brain development defect (DD) and neurodevelopmental defect (NDD) as well as unaffected controls including siblings. By employing our previously developed workflow RBP-Var, we investigated the potential implication of these \textit{de novo} mutations involved in post-transcriptional regulation in these six neuropsychiatric disorders and found that a subset of \textit{de novo} mutations could classified as piDNMs. Moreover, we observed a higher prevalence of piDNMs in the probands of neuropsychiatric disorders versus controls. In addition, gene ontology (GO) enrichment analyses of cross-disorder genes containing piDNMs in at least two of these disorders revealed several shared crucial pathways involved in the biological processes of neurodevelopment. Meanwhile, weighted gene co-expression network analysis (WGCNA) and protein-protein interactions analysis showed enriched co-expression modules and intensive protein-protein interactions between these cross-disorder genes, respectively, implying that there was convergent machinery of post-transcriptional regulation in six psychiatric disorders. Furthermore, we established an interaction network which is centered on several
RBP hubs and encompassed with piDNMs-containing genes. In short, DNMs which are deleterious to post-transcriptional regulation highly likely contribute to the pathogenesis of neuropsychiatric disorders.
Results

Our ultimate goal was to identify all piDNMs from DNM in six neuropsychiatric disorders by analyzing a variety of aspects related to post-transcriptional regulation process. To do this, we first developed a comprehensive workflow with a statistic algorithm that links the potentially functional roles in post-transcriptional regulation to DNMs. We then used this workflow to identify functional variants from 9,772 core family with 42,871 de novo mutations associated with six neuropsychiatric disorders, including autism spectrum disorder (ASD), epileptic encephalopathy (EE), intellectual disability (ID), schizophrenia (SCZ), brain development defect (DD) and neurodevelopmental defect (NDD) as well as unaffected siblings (Supplementary Table 1). We further presented an online tool which could rapidly annotates and classifies piDNMs, and determines their potential roles in neuropsychiatric disorders.

High occurrence of piDNM in neuropsychiatric disorders

First, we used our previously developed workflow RBP-Var\textsuperscript{21} to identify functional piDNMs from 9,772 trios with 42,871 de novo mutations (29,041 DNMs in probands and 13,830 DNMs in controls) across six kinds of neuropsychiatric disorders as well as their unaffected siblings (control). We determined DNMs with 1/2 categories predicted by RBP-Var as piDNMs and identified 2,381 piDNMs in probands (Supplementary Table 2). In detail, RBP-Var identified 1,410 piDNMs in ASD, 356 piDNMs in DD, 281 piDNMs in SCZ, 103 piDNMs in EE, 129 piDNMs in NDD and 102 piDNMs in ID while only 398 piDNMs were identified in controls groups (Supplementary Table 3). Interestingly, the average frequency of piDNMs in each patient in any one of six
neuropsychiatric disorders were significantly over-represented compared with the one in controls ($P = 9.52\text{E}-17$, Figure 1A). With the combined data from 9,772 trios, we also observed that probands groups have much higher odds ratio (OR) of piDNMs compared with controls in all six kinds of neuropsychiatric disorders (Figure 1B; Supplementary Table 4). Dramatically, synonymous SNVs and unknown SNVs (SNVs which located in UTRs) in piDNMs were more prevalent in neuropsychiatric disorders versus controls (Supplementary Figure 1A-B), suggesting that synonymous SNVs and UTR SNVs which do not directly change the protein composition may also play crucial roles in the pathogenesis of neuropsychiatric disorders by influencing post-transcriptional regulation. Indeed, more than 50 diseases have been reported to be associated with synonymous SNVs in human genes$^{23}$. In short, piDNMs that are linked to post-transcriptional dysregulation may contribute to the pathogenesis of these six neuropsychiatric disorders.

**Robust discrimination of risk by piDNMs**

Our platform RBP-Var determined the post-transcriptional dysfunction of piDNMs regardless of considering whether the DNMs give rise to protein truncating. To investigate the potential role of DNMs in different regulatory processes, we compared our RBP-Var$^{21}$ prediction tool with other three popular tools including SIFT$^{25}$, PolyPhen2 (PPH2)$^{26}$ and RegulomeDB$^{24}$. RBP-Var, RegulomeDB and SIFT/PPH2 were designed to evaluate the impact of mutation on post-transcriptional regulation, transcriptional regulation and protein product, respectively. We found that SIFT and PPH2 discriminated nonsynonymous/stop DNMs ($P = 5.37\text{E}-62$, $P = 4.55\text{E}-03$) and nonsynonymous/synonymous DNMs ($P = 8.76\text{E}-123$, $P < 2.20\text{E}-16$), respectively (Figure 2A, B). However, RegulomeDB discriminated nonframeshift ($P < 2.20\text{E}-16$), nonsynonymous ($P = 1.12\text{E}-39$),
splicing ($P = 7.62\text{E}-04$), stop ($P < 2.20\text{E}-16$) and synonymous ($P = 3.21\text{E}-22$) DNM}s rather than frameshift DNM}s ($P = 4.14\text{E}-01$) (Figure 2C). Moreover, RBP-Var could discriminate the deleterious DNM}s on effect of frameshift ($P = 1.38\text{E}-03$), nonsynonymous ($P = 6.31\text{E}-60$), splicing ($P < 2.20\text{E}-16$) and synonymous ($P = 8.98\text{E}-09$) (Figure 2D).

Our results indicated that both RegulomeDB and RBP-Var better distinguish deleterious DNM}s from benign ones compared to SIFT and PPH2. However, the number of deleterious DNM}s detected by RegulomeDB is much less than that of RBP-Var, suggesting that RegulomeDB has a higher false-negative rate than RBP-Var. Therefore, we performed receiver operating characteristic (ROC) analysis to systemically evaluate the sensitivity and specificity of these four prediction methods. We found that area under curve (AUC) value of SIFT, PPH2, RBP-Var and RegulomeDB are 78.27 %, 76.57 %, 82.89 % and 50.77 %, respectively (Supplementary Figure 2). Moreover, the AUC value of SIFT, PPH2, and RBP-Var is significantly more sensitive and specific higher than that of RegulomeDB with $P$ value 1.63E-10, 2.40E-08 and 2.51E-60, respectively. In addition, while the AUC value of AUC value of RBP-Var is significantly higher than that of SIFT and PPH2 with $p$ value 0.049 and 0.019, respectively. Consequently, we compared the deleterious DNM}s detected by these four methods and found that RBP-Var detected more piDNMs that overlapped with predictions of SIFT and PPH2 than that of RegulomeDB. In contrast, RBP-Var could detect an additional 1,234 piDNMs that were regarded as benign DNM}s by other three methods, accounting for 37.29% of total 3,309 deleterious DNM}s detected by all four tools (Supplementary Figure 3). In terms of genes related to deleterious DNM}s, 909 functional genes (Supplementary Figure 4) which were determined by RBP-Var specially and the gene ontology were enriched in some neurodevelopmental


pathways such as Notch signaling pathway \((P = 9.82E-4)\), Neural Crest Differentiation \((P = 0.0044)\), ErbB Signaling Pathway \((P = 0.0048)\) and Signaling Pathways in Glioblastoma \((P = 7.48E-4)\) (Supplementary Figure 5; Supplementary Table 5). Whereas, the functional genes detected specifically by RBP-Var in controls were not enriched in any gene ontology. Therefore, the piDNMs detected by RBP-Var are distinct and may play significant roles in the post-transcriptional processes of the development of neuropsychiatric disorders.

**Distinct features of piDNM between disorders**

Our previous study using the NPdenovo database demonstrated that *de novo* mutations predicted as harmful in protein level are shared by four neuropsychiatric disorders\(^25\). Therefore, we wondered whether there were common piDNMs among six neuropsychiatric disorders. Firstly, we identified 24 genes harboring recurrent piDNMs among 2,381 piDNMs, including nine genes from ASD trios, 10 genes from DD&NDD trios, six genes from ID trios, and one gene from EE trios (Figure 3A; Supplementary Table 6). Only two genes harbored cross-disorder recurrent piDNMs. One was STXBP1 which occurred in both DD and EE, and another was CTNNB1 which occurred in both ASD and DD. However, we identified 312 genes carrying at least two piDNMs among 1,923 candidate genes (accounting for 16.22%), including 252 genes from ASD trios, 38 genes from EE trios, 40 ID trios, 68 SCZ trios and 132 genes from DD&NDD trios by RBP-Var (Figure 3B; Supplementary Table 7).

Subsequently, we compared the genes harboring piDNMs across six disorders and found 214 genes significantly shared by at least two kinds of disorders rather than random overlaps (permutation test, \(P < 1.00E-5\) based on random resampling, Figure 3C, D). Furthermore, the genes harboring piDNMs in controls also have
significantly fewer overlaps with the 214 shared genes in the six disorders than random overlaps ($P = 0.007$, Supplementary Figure 6A).

In addition, the number of shared genes for any pairwise comparison is significantly higher than random overlaps ($P < 0.05$) except for the comparison of EE versus SCZ ($P = 0.17864$) (Supplementary Figure 7). On the contrary, the genes harboring piDNMs in controls have significantly fewer overlaps with disorders than random overlaps: ASD ($P < 1.00E-5$, Supplementary Figure 6B), ID ($P < 1.00E-5$, Supplementary Figure 6C), EE ($P = 1.20E-4$, Supplementary Figure 6D), SCZ ($P < 1.00E-5$, Supplementary Figure 6E) and DD&NDD ($P < 1.00E-5$, Supplementary Figure 6F). Our observation suggests that there are common genes harboring piDNMs in these six neuropsychiatric disorders.

Several neuropsychiatric disorders share many symptoms suggesting common underlying molecular mechanism exist that may implicate important regulators of pathogenesis. Thus, we performed clueGO analysis for these shared genes and found they were enriched in crucial neurodevelopmental pathways (Supplementary Table 8; Supplementary Figure 8), including thyroid hormone signaling pathway ($q = 1.10E-5$), modulation of synaptic transmission ($q = 4.50E-5$), motor activity ($q = 6.10E-5$), dopamine receptor binding ($q = 1.80E-4$), visual learning ($q = 2.00E-4$), cognition ($q = 3.00E-4$), learning or memory ($q = 2.00E-3$), postsynaptic density ($q = 2.40E-03$), regulation of GABAAergic synaptic transmission ($q = 5.90E-03$), regulation of synaptic plasticity ($q = 1.00E-2$), regulation of axonogenesis ($q = 2.20E-2$), cell cortex ($q = 2.20E-2$) and regulation of oligodendrocyte differentiation ($q = 4.80E-2$). Intriguingly, we found some enriched epigenetic pathways, such as epigenetic regulation of gene expression ($q = 3.50E-8$), histone methylation ($q = 4.60E-4$), histone lysine methylation ($q = 3.7E-3$), macromolecule methylation ($q = 1.10E-2$), chromatin silencing ($q = 1.10E-2$), chromatin
remodeling ($q = 1.20 \times 10^{-2}$), regulation of chromatin organization ($q = 1.40 \times 10^{-2}$), chromatin-mediated maintenance of transcription ($q = 1.60 \times 10^{-2}$). These epigenetic regulating genes are composed of ARID1B ($P_{\text{TADA}} = 2.29 \times 10^{-8}$), CTCF ($P_{\text{TADA}} = 2.29 \times 10^{-8}$), CTNNB1 ($P_{\text{TADA}} = 2.29 \times 10^{-8}$), EP300 ($P_{\text{TADA}} = 2.29 \times 10^{-8}$), MECP2 ($P_{\text{TADA}} = 4.58 \times 10^{-7}$), DNMT3A ($P_{\text{TADA}} = 7.33 \times 10^{-7}$), KMT2D ($P_{\text{TADA}} = 1.86 \times 10^{-4}$), MYO1E ($P_{\text{TADA}} = 8.80 \times 10^{-4}$), CNOT1 ($P_{\text{TADA}} = 6.05 \times 10^{-3}$), TNRC18 ($P_{\text{TADA}} = 6.80 \times 10^{-3}$), JARID2 ($P_{\text{TADA}} = 1.70 \times 10^{-2}$), PHF19 ($P_{\text{TADA}} = 7.54 \times 10^{-2}$), TNRC6A ($P_{\text{TADA}} = 9.31 \times 10^{-2}$), DOT1L ($P_{\text{TADA}} = 1.68 \times 10^{-1}$), KMT2B ($P_{\text{TADA}} = 5.64 \times 10^{-1}$) (Supplementary Table 9). Moreover, most of these epigenetic modification genes, especially for genes with $P_{\text{TADA}} < 0.05$, have been previously linked with neuropsychiatric disorders$^{26-29}$. Interestingly, shared genes in clueGO enrichment analyses are intensive linkages among these significant pathways as some of piDNMs-containing genes could play roles in more than one of these pathways (Supplementary Figure 9).

In contrast, we also observed hundreds of piDNM-containing genes are disorder-specific (Figure 3C). Follow-up GO enrichment analysis revealed that 226, four and eight GO terms were enriched for ASD, SCZ and DD&NDD from these disorder-specific genes ($q < 0.05$), respectively. Several GO terms are associated with neurodevelopmental defects. For example, GO terms “neurogenesis” ($q = 6.70 \times 10^{-3}$) and “neuron development” ($q = 2.70 \times 10^{-2}$) were enriched for ASD-specific genes (Supplementary Table 10). And GO terms “regulation of autophagy” ($3.00 \times 10^{-3}$), “regulation of macroautophagy” ($3.40 \times 10^{-3}$), “macroautophagy” ($1.60 \times 10^{-2}$) and “regulation of establishment of planar polarity involved in neural tube closure” ($1.80 \times 10^{-2}$) were enriched in SCZ-specific genes (Supplementary Table 11). In addition, GO terms “response to UV” ($q = 2.20 \times 10^{-3}$), “regulation of chromatin organization” ($q = 8.50 \times 10^{-3}$), “helicase activity” ($q = 1.00 \times 10^{-2}$), “histone methyltransferase complex” ($q = 1.40 \times 10^{-2}$), “cellular response to UV” ($q = 1.70 \times 10^{-2}$).
02), “viral genome replication” (q = 1.90E-02) and “regulation of histone acetylation” (q = 2.30E-02) were enriched in DD&NDD specific genes (Supplementary Table 12). However, there was no enriched GO terms for ID and EE specific genes. These disorder-specific genes may point to disorder-specific phenotypes and distinctive biological processes among the six neuropsychiatric disorders.

**Enriched co-expression modules**

As co-expression of genes has been used to explore the common and distinct molecular mechanism in neuropsychiatric disorders\(^3^0\), we performed weighted gene co-expression network analysis (WGCNA)\(^3^1\) with 214 cross-disorder transcripts by using up-to-date BrainSpan developmental transcriptome (n=524): gene expression in 16 human brain structures across 31 developmental stages\(^3^2\). The results of WGCNA deciphered two gene modules with distinct spatiotemporal expression patterns (Figure 4A, B; Supplementary Figure 10). The turquoise module (n=146 genes) is characterized by high expression during early fetal development (8-37 postconceptional weeks) in most of brain structures (Figure 4C). The blue module (n=58 genes) is delineated to low expression in early fetal development (postconceptional week 8) and early childhood (3 year) in most of brain structures (Figure 4D). Only two out of the 214 cross-disorder genes were not clustered in the co-expression networks (Supplementary Table 13). In addition, we constructed the co-expression network by using gene pairs with correlation weight of at least 0.3 (Supplementary Table 14). Interestingly, we found that majority of the co-expression hubs were histone modifiers, such as KAT6B, KDM5B, SETDB1, TRRAP, EP300, and transcriptional regulators, including CTCF, SMARCA4, ARID1B, ADNP, CHAMP1 (Supplementary Figure 11). Consequently, the link among these six highly heterogeneous neuropsychiatric disorders...
disorders may be represented by biological processes controlled by these hub genes in co-expression networks.

Furthermore, GO enrichment analysis revealed distinct biological processes within the two modules. The turquoise module was enriched for epigenetic regulation of gene expression (q = 3.99E-08), establishment or maintenance of cell polarity (q = 8.24E-04), Notch binding (q = 1.33E-3), Thyroid hormone signaling pathway (q = 1.75E-3), beta-catenin-TCF complex assembly (q = 5.81E-3), chromatin remodeling (q = 1.30E-2), Notch signaling pathway (q = 2.51E-2), motor activity (q = 3.21E-2) and other enriched GO terms (Supplementary Figure 12; Supplementary Table 15). The blue module was enriched visual learning (q = 4.92E-07), regulation of synaptic plasticity (q = 1.91E-06), actin filament-based movement (q = 6.52E-06), adult locomotory behavior (q = 3.86E-04), neuromuscular process (q = 8.61E-04), Long-term depression (q = 1.02E-3), positive regulation of dendrite development (q = 1.35E-3), negative regulation of autophagy (q = 3.74E-3), negative regulation of G-protein coupled receptor protein signaling pathway (q = 5.07E-3) and other neurodevelopment related pathways (Supplementary Figure 13; Supplementary Table 16).

**Intensive protein-protein interactions**

The co-expression results indicated that these 214 cross-disorder proteins may have intensive protein-protein interactions (PPI). To identify common biological processes that potentially contribute to disease pathogenesis, we established protein-protein interactions within these 214 cross-disorder genes by investigating protein-protein interactions curated in database BioGRID. Surprisingly, 128 out of 214 (59.81%) cross-disorder genes represent an interconnected network on the level of available direct/genetic protein-protein interactions (Figure 5A). In...
addition, an overwhelming majority (98.54%, 203 out of 206 pairs) of the protein-protein interactions constitute direct interactions while only 3 interaction pairs are genetic interactions (Supplementary Table 17). Furthermore, we determined several crucial hubs of protein-protein interactions, including CUL3, CTNNB1, HECW2, HNRNPU, EP300, SMARCA4, ARRB2, PTPRF, MYH9 and NOTCH1 (Figure 5A), which may control common biological processes among these six neuropsychiatric disorders. Indeed, these 128 cross-disorder proteins are enriched in nervous system phenotypes, including abnormal synaptic transmission, abnormal nervous system development, abnormal neuron morphology and abnormal brain morphology, and behavior/neurological phenotype such as abnormal motor coordination/balance (two sided Fisher's Exact Test, q < 0.05, Supplementary Figure 14A). Similarly, these 128 genes in interaction network are enriched in nervous system phenotype including abnormal nervous system development and abnormal brain morphology (two sided Fisher's Exact Test, q < 0.05, Supplementary Figure 14B). Dramatically, HNRNPU belongs to the subfamily of ubiquitously expressed heterogeneous nuclear ribonucleoproteins (hnRNPs) which are RNA binding proteins and they form complexes with heterogeneous nuclear RNA (hnRNA). These proteins are associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport.

To investigate the expression of these co-interacting genes in the human cortex of ASD patients and normal donors, we downloaded and compared the normalized FPKM value from Gene Expression Omnibus (GEO) with accession number GSE64018 and GSE76852, respectively. We identified 97 significantly differential expression genes (accounting for 75.78% of the PPI genes) between ASD patients and normal controls (Student's t-test, q < 0.05, Figure 5B). And 86 of
these PPI genes were highly expressed in ASD patients while only 11 genes were
down-regulated in ASD patients when compared with normal controls
(Supplementary Table 18), implying that most of these PPI genes were abnormally
expressed in ASD patients.

**Shared networks between piDNM and RBPs**

According to previous studies, dysregulation or mutations of RBPs can cause
a range of developmental and neurological diseases. By contrast, mutations in
RNA targets of RBPs, which could disturb the interaction between RBPs and their
mRNA targets, would affect mRNA metabolism and protein homeostasis in
neurons by impair RNA transport and translation in neuropathological disorders.
Indeed, mutations that alter sequence context of RBP binding sites commonly
affect RBP binding and regulation. For instance, regulation depended on FMRP-
related activity during fetal brain development might be particularly vulnerable to
genetic perturbations, with severe mutations resulting in disruption of
developmental canalization. Hence, we constructed a regulatory network between
piDNMs and RBPs to investigate the genetic perturbations of mRNA-RBP
interactions in six disorders (Figure 6). We identified several crucial common RBP
hubs, including EIF4A3, FMR1, PTBP1, AGO1/2, ELAVL1, IGF2BP1/3, WDR33
and FXR2. Genes with piDNM in different disorders could be regulated by the
same RBP hub while one candidate genes may be regulated by different RBP hubs
(Figure 6). In addition, all of these RBP hubs were highly expressed in early fetal
development stages (8-37 postconceptional weeks) based on BrainSpan
developmental transcriptome (Supplementary Figure 15), suggesting that these
RBP hubs may play important roles in the early stages of brain development.

**The PostOmics database**
To make our findings easily accessible to the research community, we have developed RBP-Var2 platform (http://www.rbp-var.biols.ac.cn/) for storage and retrieval of piDNMs, candidate genes, for exploring the genetic etiology of neuropsychiatric disorders in post-transcriptional regulation.

Discussion

In this study, we systematically analyzed the damaging effect of de novo mutations at the level of post-transcriptional regulation in six neuropsychiatry disorders. We discovered 2,381 piDNMs that were extremely deleterious on post-transcriptional regulation, of which 1,034 (43.43%) piDNMs were extremely deleterious (predicted by NPdenovo) on the structure and function of protein. Among the total 1,923 genes containing piDNMs, 862 (44.83%) genes were predicted to simultaneously alter the structure and function of correspondingly coded proteins. On the other hand, 1007 out of 2672 (37.69%) genes and 1023 of 3714 (27.54%) extreme DNMs predicted by NPdenovo were also extremely deleterious predicted by RBP-Var. Our observations indicate that over half of piDNMs are not expect to affect the structure and function of protein but are predicted to affect post-transcriptional regulation, and vice versa. Moreover, probands have higher odds ratio of piDNMs than controls in all kinds of neuropsychiatric disorders. Therefore, piDNMs are one of major contributors to etiology for neuropsychiatry disorders.

We applied RBP-Var algorithm to annotate and interpret de novo variants in subjects with six neuropsychiatry disorders and control subjects. We observed a strong enrichment of pathogenic or likely pathogenic variants in affected subjects. In comparison with accuracy of RBP-Var (82.89%), other prediction algorithms such as SIFT or PPH2 have comparative accuracy (76~78%) while RegulomeDB
has much lower accuracy (50.77%) to differentiate affected from control subjects. This observation suggests that the potential pathogenicity of genetic variants is mainly due to post-transcriptional dysregulation and malfunction of protein, but not disruption of transcription process. Therefore, deciphering multiple biological layers of deleteriousness, particularly post-transcriptional regulation and structure/function of protein, may improve the accuracy of algorithm’s prediction.

To date, it has been a challenge to estimate the deleteriousness of synonymous and UTRs mutations though such mutations have been widely acknowledged alter protein expression, conformation and function⁴¹. Nevertheless, our data-based algorithm identified 518 synonymous DNMs and 82 UTR’s DNMs were extremely deleterious in post-transcriptional regulation. Moreover, synonymous damaging DNMs were significantly prominent in probands compared with that of controls, supported by predictions of both RBP-Var and RegulomeDB. Meanwhile, de novo insertions and deletions (InDels), especially frameshift patterns are taken for granted to be deleterious. Indeed, de novo frameshift InDels are more frequent in neuropsychiatry disorders compared to non-frameshift InDels⁴², which were demonstrated by predictions of RBP-Var but not SIFT and PPH2 (Figure 2). To our knowledge, our research is the first investigation into the post-transcriptional etiology of de novo non-frameshift InDels and de novo synonymous mutations as well as de novo protein-truncating variants in neuropsychiatry disorders.

Most interestingly, we discovered some enriched epigenetic pathways, such as those that regulate gene expression and histone methylation. This finding is consistent with a previous report that demonstrated that more than 68% of ASD cases shared a common acetylome aberrations at >5,000 cis-regulatory regions in prefrontal and temporal cortex⁴³. Such common "epimutations" may be induced by either perturbations of epigenetic regulations (including post-transcriptional
regulations) due to genetic mutations of substrates or the disruptions of epigenetic modifications due to the genetic mutation of epigenetic genes. Actually, our observations suggest that alterations of "epimutations" were associated with the dysregulation of post-transcription. In fact, we found several recurrent piDNM-containing genes including CHD8, CHD2, SYNGAP1, ADNP, POGZ, ANK2 and DYRK1A, are non-epigenetic genes. Moreover, we also discovered several recurrent epigenetic genes including KMT2A, KMT2B, KMT2C, KAT6B, KDM3B, JARID2, DNMT3A and MECP2, that contain piDNMs which may play important roles in the genome-wide aberrations of epigenetic landscapes through disruption the post-transcription regulation. Furthermore, WGCNA analysis revealed that major hubs of the co-expression network for these 214 piDNM-containing genes were histone modifiers by using BrainSpan developmental transcriptome. These data indicate that piDNM-containing genes are co-expressed with genes frequently involved in epigenetic regulation of common cellular and biological process in neuropsychiatric disorders.

Importantly, these 214 piDNMs-containing genes harbor intensive protein-protein interactions in physics and shared regulatory networks between piDNMs and RBPs in six neuropsychiatric disorders. We identified several RBP hubs of regulatory networks between piDNMs-containing genes and RBP proteins, including EIF4A3, FMRP, PTBP1, AGO1/2, ELAVL1, IGF2BP1/3, WDR33 and FXR2. Taking FMRP for example, it is a well-known pathogenic gene of Fragile X syndrome which co-occurs with autism in many cases and its targets are highly enriched for de novo mutations in ASD. The mutation of any RBP hubs may result in multiple disorders and mutations of regulated genes may disrupt interactions with multiple RBPs. Fortunately, we have constructed the regulatory networks among multiple RBPs and their target RNAs with de novo mutations that
could significantly disturb the regulatory networks in six neuropsychiatric disorders. Thus, we provided a framework for interpreting network biology research and leveraging big genomics data sets in neurobiology of post-transcriptional regulation.

Alterations in expression or mutations in either RBPs or their binding sites in target transcripts have been reported to be the cause of several human diseases such as muscular atrophies, neurological disorders and cancer\(^4^4\). However, it is still a challenge to decipher the effect of genetic mutation on RNA and post-transcriptional regulation and additional clinical and molecular datasets are required to improve the sensitivity and specificity of our data and algorithm based platform. However, our method sheds light on evaluation of post-transcriptional impact of genetic mutations including synonymous mutations which frequently act as driver mutations in human cancers\(^4^5\). In addition, small molecules can be rapidly designed to selectively target RNAs and affect RNA-RBP interactions, leading to anti-cancer strategies\(^4^6\). Therefore, the discovery of disease-causing mutations in RNAs is yielding a wealth of new therapeutic targets, and the growing understanding of RNA biology and chemistry is providing new RNA-based tools for developing therapeutics\(^4^7\).
Materials and methods

Data collection.

For this study, 9,772 trios were recruited from previous WES/WGS studies, comprising 7,453 parent-probands trios associated with six neuropsychiatric disorders and 2,319 control trios. After deduplication, a total of 29,041 DNMs in probands and 13,830 DNMs in controls were identified for subsequent analysis.

Identification of deleterious piDNMs and comparison with variants predicted by other methods.

To determine the likelihood of causing a deleterious mutation in post-transcriptional regulation for all SNVs and InDels, our previously developed program RBP-Var\textsuperscript{48} was utilized to assign an exclusive rank for each mutation and only those mutations categorized into rank 1 or 2 were considered as piDNMs. In comparison with those mutations involved in the disruption of gene function or transcriptional regulation, several programs such as SIFT, PolyPhen2 and RegulomeDB were used to analyze the same dataset of DNMs as the input for RBP-Var. We only kept the mutations qualified as “damaging” from the result of SIFT and “possibly damaging” or “probably damaging” from PolyPhen2. In the case of RegulomeDB, mutations labelled as category 1 and 2 were retained. Next, we classified the type of mutation (frameshift, nonframeshift, nonsynonymous, synonymous, splicing and stop) and located regions (UTR3, UTR5, exonic, ncRNA exonic and splicing) in order to determine distribution of piDNMs, genetic variants and other regulatory variants. The number of variants in cases versus controls was illustrated by bar chart (\(***: P < 0.001, **: 0.001 < P < 0.01, *: 0.01 < P < 0.05\), binomial test).
**TADA analysis of DNMs in six disorders**

The TADA program (Transmission And De novo Association), which predicts risk genes accurately on the basis of allele frequencies, gene-specific penetrance, and mutation rate, was used to calculate the P-value for the likelihood of each gene contributing to the all six disorders with default parameters.

**ROC curves and specificity/sensitivity estimation**

We screened a positive (non-neutral) test set of likely casual mutations in Mendelian disease from the ClinVar database (v20170130). From a total of 237,308 mutations in ClinVar database, we picked up 145 exonic mutations presenting in our curated DNMs in probands. Our negative (neutral) set of likely non-casual variants was built from DNMs of unaffected siblings in six neuropsychiatric disorders. In order to avoid rare deleterious DNMs, we selected only DNMs in controls with a minor allele frequency of at least 0.01 in 1000 genome (1000g2014oct), resulting in a set of 921 exonic variants. Then, we employed R package pROC to analyze and compare ROC curves.

**Overlaps of piDNMs between disorders.**

In order to elucidate the overlap of genes among any two of the six disorders as well as each disorder and the control, we shuffled the intersections of genes and repeated this procedure 100,000 times. During each permutation, we randomly selected the same number of genes as the actual situation from the entire genome for each disorder and the control, then p values were calculated as the proportion of permutations during which the observed number of genes was above/below the number of genes in simulated situations.

**Functional enrichment analysis**
A gene harboring piDNMs would be selected into our candidate gene set to conduct functional enrichment analysis if it occurred in at least two of the six disorders. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichments analysis was implemented by Cytoscape (version 3.4.0) plugin ClueGO (version 2.3.0) and $P$ values calculated by hypergeometric test was corrected to be $q$ values by Benjamini–Hochberg procedure for reducing the false discovery rate resulted from multiple hypothesis testing.

**Co-expression and spatiotemporal specificity**

Normalized gene-expression of 16 human brain regions were determined by RNA sequencing and obtained from BrainSpan (http://www.brainspan.org). Expression for 206 out of 214 extreme damaging cross-disorder genes was extracted and clustered according to their expression pattern by using R-package WGCNA (weighted correlation network analysis). The expression level for each gene and development stage (only stages with expression data for all 16 structures were selected, $n = 14$) was presented across all brain regions.

**Protein-protein interaction and phenotype enrichment**

Cross-disorder genes containing piDNMs were subjected to esyN analysis for generating a network of protein interactions and the network of protein interaction was created by using physical and genetic interactions of H. sapiens curated in BioGRID. Cytoscape (version 3.4.0) was used to analyze and visualize protein-protein interaction networks. Overrepresentation of mouse-mutant phenotypes was evaluated using the web tool MamPhea for the genes in the PPI network and for all cross-disorder genes containing piDNMs. Rest of genome was used as background and $q$ values was calculated from $p$ values by Benjamini–Hochberg correction.

**Gene-RBP interaction network**
Cytoscape (version 3.4.0) was utilized for visualization of the associations between genes harboring piDNMs in the six neuropsychiatric disorders and the corresponding regulatory RBPs.

**URLs**


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**Conflict of interest**

The authors declare that there are no conflicts of interest regarding to publishing this paper.

**Figure legends**

**Figure 1.** The abundance of DNMs in different RBP-Var categories. (A) The quantities of DNMs in patients with neuropsychiatric disorders are significantly
larger than that in normal controls, shown in several categories classified by RBP-Var. (B) The horizontal bar plot corresponds to the odds ratios indicating the enrichment of piDNMs in patients with any of the five neuropsychiatric disorders. The bar plot of normal controls corresponds to the odds ratio of 1 with no enrichment.

**Figure 2.** Performance comparison of the ability to distinguish severe DNMs between RBP-Var and three other tools. (A) Different kinds of DNMs affecting protein function predicted by SIFT. The Y-axis corresponds to the proportion of each kind of mutations within the total number of damaging DNMs predicted by SIFT. (B) Different kinds of DNMs that affect protein function predicted by PolyPhen2. The Y-axis corresponds to the proportion of each kind of mutations within the total number of damaging DNMs predicted by PolyPhen2. (C) The DNMs predicted as functional elements involved in transcriptional regulation by RegulomeDB are categorized into different functional types. The Y-axis corresponds to the proportion of each kind of mutations within the total number of damaging DNMs predicted by RegulomeDB. (D) The DNMs classified as either level 1 or 2 (piDNMs) are categorized into different functional types. The Y-axis corresponds to the proportion of each kind of mutations within the total number of damaging piDNMs. The P values were measured by two-sided binomial test.

**Figure 3.** Candidate genes selected by RBP-Var involved in six neuropsychiatric disorders. (A) Scatter plot of 24 genes harboring recurrent piDNMs among 2381 piDNMs. The Y-axis corresponds to the –log10(P value) calculated by TADA. The X-axis stands for the TADA output of –log10(mutation rate). (B) Scatter plot of 312 recurrent genes among 1,923 candidate genes. The Y-axis corresponds to the –log10(P value) calculated by TADA. The X-axis stands for the TADA output of –log10(mutation rate). (C) Venn diagram representing the distribution of candidate
genes shared among five neuropsychiatric disorders. (D) Permutation test for the validity of the overlap between the candidate genes involved in the five neuropsychiatric disorders. We shuffled the genes of each disorder and calculated the shared genes between the five disorders, repeating this procedure for 100,000 times to get the null distribution. The vertical dash line stands for the observed value corresponding to a P value of the permutation test.

**Figure 4.** Weighted co-expression analysis of 214 shared genes. (A) Heat map visualization of the co-expression network of 214 shared genes. The more saturated color corresponds to the highly expressed genes. (B) Hierarchical clustering dendrogram of the two color-coded gene modules displayed in (A). (C, D) Heat maps of the expression of the two respective gene modules.

**Figure 5.** Protein-protein interaction network analysis. (A) The network of interactions between pairs of proteins of 128 out 214 shared genes. (B) Heat map showing significantly differential expression of 97 out of 128 genes involved in the protein-protein interaction network.

**Figure 6.** Interaction network of RBPs and genes with piDNMs. Different roles of the nodes are reflected by distinguishable geometric shapes and colors. The magenta vertical arrow stands for the RNA binding proteins. Disks with different colors represent the genes with piDNMs involved in different kinds of disorders.

odds ratios of piDNMs in the forms of insertion that involved in the six neuropsychiatric disorders relative to the normal controls.

**Supplementary Figure 1.** Odds ratio of different functional types of piDNMs. (A-G) Bar plot of the odds ratios of piDNMs in various functional types involved in the six neuropsychiatric disorders relative to the normal controls.
Supplementary Figure 2. ROC curve showing the performance of the predictions of SIFT, PPH2, RBP-Var and RegulomeDB.

Supplementary Figure 3. Venn diagram depicting the overlap between the DNMs predicted by SIFT, PPH2, RBP-Var and RegulomeDB.

Supplementary Figure 4. Venn diagram depicting the overlap between the genes predicted by SIFT, PPH2, RBP-Var and RegulomeDB.

Supplementary Figure 5. Pathway enrichment analysis of the 909 genes unique to the prediction of RBP-Var.

Supplementary Figure 6. Test of the significance of the genes shared between each pair of the five disorders. (A-G) Permutation test for the validity of the gene overlap between each pair of the five disorders. We shuffled the genes of each disorder and calculated the shared genes between each pair, repeating this procedure for 100,000 times to get the null distribution. The vertical dash line stands for the observed value corresponding to a P value of the permutation test.

Supplementary Figure 7. Test of the significance of the 214 candidate genes involved in the five neuropsychiatric disorders. (A-E) Permutation test for the validity of the gene overlap between each disorder and the normal control. (F) Permutation test for the validity of the gene overlap between the 214 shared genes and the normal control.

Supplementary Figure 8. Pie chart of the pathway enrichment analysis for the 214 shared genes.

Supplementary Figure 9. Interaction network of the gene enrichment analysis for the 214 shared genes.
Supplementary Figure 10. Relationship between Co-expression modules. (A) MDS plot of genes in turquoise module and blue module. (B) Relationship between module eigengenes. (C) Clustering tree based of the module eigengenes. (D) heatmap of adjacency Eigengene.

Supplementary Figure 11. The co-expression network of 206 out of the 214 shared genes. Different size of the node is representative of the number of connections between the gene and others.

Supplementary Figure 12. Pie chart showing the enrichment analysis of the genes clustered in the turquoise module from the weighted co-expression analysis.

Supplementary Figure 13. Pie chart showing the enrichment analysis of the genes clustered in the blue module from the weighted co-expression analysis.

Supplementary Figure 14. Mammalian phenotype enrichment analysis of selected genes. (A) Mammalian phenotype enrichment of 214 cross-disorder piDNMs genes. (B) Mammalian phenotype enrichment of 128 genes in interaction network.

Supplementary Figure 15. Heat map of the expression of the crucial RBP hub genes during the early fetal development stages.
Reference


