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Low-Level Brain Somatic Mutations Are Implicated in Schizophrenia

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ABSTRACT

BACKGROUND: Somatic mutations arising from the brain have recently emerged as significant contributors to neurodevelopmental disorders, including childhood intractable epilepsy and cortical malformations. However, whether brain somatic mutations are implicated in schizophrenia (SCZ) is not well established.

METHODS: We performed deep whole exome sequencing (average read depth $>$ 550$\times$) of matched dorsolateral prefrontal cortex and peripheral tissues from 27 patients with SCZ and 31 age-matched control individuals, followed by comprehensive and strict analysis of somatic mutations, including mutagenesis signature, substitution patterns, and involved pathways. In particular, we explored the impact of deleterious mutations in *GRIN2B* through primary neural culture.

RESULTS: We identified an average of 4.9 and 5.6 somatic mutations per exome per brain in patients with SCZ and control individuals, respectively. These mutations presented with average variant allele frequencies of 8.0% in patients with SCZ and 7.6% in control individuals. Although mutational profiles, such as the number and type of mutations, showed no significant difference between patients with SCZ and control individuals, somatic mutations in SCZ brains were significantly enriched for SCZ-related pathways, including dopamine receptor, glutamate receptor, and long-term potentiation pathways. Furthermore, we showed that brain somatic mutations in *GRIN2B* (encoding glutamate ionotropic NMDA receptor subunit 2B), which were found in two patients with SCZ, disrupted the location of GRIN2B across the surface of dendrites among primary cultured neurons.

CONCLUSIONS: Taken together, this study shows that brain somatic mutations are associated with the pathogenesis of SCZ.

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With a prevalence of ~1% worldwide, schizophrenia (SCZ) is a neurodevelopmental disorder characterized by delusion, hallucination, and cognitive impairment (1). SCZ is also known to be associated with neural network dysfunction and abnormal gene expression in the dorsolateral prefrontal cortex (DLPFC) (2–5). Regarding disease etiology, genetic variants are thought to be major contributors to SCZ (6), and previous genetic studies have documented SCZ-associated or -causing germline variations using large-scale whole exome or genome sequencing and genome-wide association studies (7–9). These germline mutations have been shown to be functionally linked to NMDA receptors, synapse, immunity, and central nervous system development (10). Notwithstanding, although previous studies have been able to describe important contributions of germline mutations to SCZ (9,11–13), germline mutations in SCZ-relevant genes and loci account for only 3.4% to 23% of all sequenced patients with SCZ (8,14–16), and a substantial portion of patients with SCZ remain genetically unexplained.

Unlike germline mutations that occur in sperm or eggs and are inherited from parents to their offspring, somatic mutations arise after fertilization and can exist as mosaic patterns in the whole body or present within localized tissues at very low variant allele frequencies (VAFs) (17,18). Such mutations are likely to go undetected even in advanced genetic analysis of blood, saliva, or other peripheral tissues. However, we and other groups have recently found that low-level brain somatic mutations indeed exist and contribute to various neurological disorders, including intractable childhood epilepsy and Alzheimer's disease (AD), of previously unknown genetic etiology (19–27). For example, brain somatic mutations in mTOR pathway genes present at VAFs less than 1% in focal cortical regions were shown to be sufficient to cause spontaneous behavioral seizures and neuronal abnormalities representative of focal cortical dysplasia (19,20). In addition, Fullard *et al.* performed whole exome sequencing (WES) of 9 patients with SCZ and 10 control individuals with read depths of 250$\times$ in brain and 50$\times$ in matched muscle tissues. This low depth of WES detected only 25 somatic single nucleotide variations (SNVs) in SCZ brain tissues, which are too few to probe the association of brain somatic mutations with SCZ (28). So far, how brain somatic mutations are associated with SCZ has remained elusive.

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Here, we performed deep WES in matched brain (DLPFC) and peripheral (mostly liver) tissues from 27 patients with SCZ and 31 age-matched control individuals. To accurately identify low-level somatic mutations in the brain, we applied high-depth sequencing (average throughput depth 569.7×) and further validated random brain somatic mutations using independent ultradeep targeted amplicon sequencing (TASeq) (749452.4×) and Sanger sequencing. We then sought to document the engagement of noted brain somatic mutations in SCZ pathogenesis by analyzing significant biological pathways enriched from genes with putatively deleterious somatic mutations. We further validated the biological function of a recurrently mutated gene in cultured primary neurons. Taken together, our results highlight the contributions of brain somatic mutations to the pathogenesis of SCZ.

METHODS AND MATERIALS

Subject Ascertainment

The DNA from brain (DLPFC, Brodmann area 46) and matched peripheral (liver or spleen) tissues of 27 patients with SCZ and 26 age-matched control individuals was provided by the Stanley Medical Research Institute (SMRI). In addition, fresh frozen human brain (DLPFC, Brodmann area 9) and matched peripheral (heart or liver) tissues of 5 control individuals were provided by the National Institute of Child Health and Human Development. Clinical information for the individuals enrolled in this study is listed in Table S1 in Supplement 2.

Deep WES

Samples were prepared according to the Agilent library preparation protocol (Agilent Human All Exon V4/V5+UTR 50-Mb kit; Agilent Technologies, Santa Clara, CA) by the SMRI. Most libraries underwent paired-end deep WES using an Illumina HiSeq 2500 instrument (Illumina, San Diego, CA) according to the manufacturer's protocol. Detailed processes that we used are provided in the Supplemental Methods in Supplement 1.

Simulating Low-Level Mutations Calling Through In Silico Mixing and Variant Calling Using MuTect and Mutect2

Detailed methods and results are described in Supplement 1.

Quality Controls

To improve the accuracy of analysis, we applied quality control processes to analysis-ready BAM files such as ContEst and global imbalance value scores. Details on the quality control steps are provided in the Supplemental Methods in Supplement 1.

Somatic SNV Calling

With BAM files that passed quality control, we ran MuTect version 1.1.7 (http://www.broadinstitute.org/cancer/cga/mutect) to detect de novo somatic mutations. Detailed methods are provided in the Supplemental Methods in Supplement 1.

Targeted Amplicon Sequencing

To acquire the precision of our strict filtering criteria, we performed TASeq. Details on amplicon sequencing and validation are provided in the Supplemental Methods in Supplement 1.

Bioinformatic Analysis

Detailed methods for analyzing mutational signature, gene set enrichment, protein–protein interactions, and the number of previous studies related to SCZ are described in the Supplemental Methods in Supplement 1.

Defining Deleterious Mutations

We defined deleterious mutations through previously developed tools known to be reliable. Detailed requirements for defining deleterious mutations are described in the Supplemental Methods in Supplement 1.

Cloning and Mutant Construction

pCIG-SEP:GRIN2B-IRES-tdTomato or pCAG-SEP:GRIN2B was generated by inserting SEP-tagged rat complementary DNA from pCI-SEP:GRIN2B (cat# 23998; Addgene, Watertown, MA) into pCIG-empty-IRES-tdTomato or DsRed-removed pCAG-DsRed (cat# 6908; Addgene). Details for cloning and mutagenesis are provided in the Supplemental Methods in Supplement 1.

Whole Cell Lysate Assay, Cell Surface Biotinylation Assay, and Western Blot

To quantify the total expression or surface expression of GRIN2B mutants in human embryonic kidney 293T cells, we performed western blot analysis with whole cell lysates and surface biotinylated samples. Details on the in vitro experiments are provided in the Supplemental Methods in Supplement 1.

Primary Culture, Immunocytochemistry, and Image Analysis

To explore the effect of mutations detected in GRIN2B on neurons, we performed primary culture and immunocytochemistry.

Germline Mutation and Mosaicism Calling

To determine whether any verified single nucleotide polymorphisms or reported SNVs were present in the study subjects, we employed the use of GATK-HaplotypeCaller version 3.5 and Mutect2 version 4.1.2.0. Details on calling germline mutations and mosaicism are provided in the Supplemental Methods in Supplement 1.

RESULTS

Detection of Low-Level Somatic Mutations in Matched Brain and Peripheral Tissues From Patients With SCZ and Control Individuals

We collected genomic DNA from 58 postmortem DLPFC (Brodmann area 9/46) and matched peripheral (mostly liver) tissues from 27 patients with SCZ and 31 age-matched control individuals (Table S1 in Supplement 2). None of the cases had a diagnosis or history of epilepsy or other neurological

neurodegenerative disorders. We then performed high-depth WES with average sequencing depths of 567.0× and 572.4× in brain and peripheral tissues, respectively (Figure S3A in Supplement 1). Next, we applied multiple quality control measures to ensure the accurate detection of low-level somatic mutations frequently confused with sequencing artifacts (29–31) (Figure S3B–D; details in Supplement 1). As a result, we were able to analyze somatic mutations in 93.1% (54/58) of the brain samples and 70.7% (41/58) of the peripheral samples (37 liver, 2 spleen, and 2 heart tissues) from 51 subjects. In addition, we confirmed that there was no correlation between postmortem interval or potential of hydrogen (pH) of brain and SNVs (Figure S3F, G in Supplement 1).

We used MuTect to detect somatic mutations because we found that MuTect showed better performance for detecting low-level somatic mutations than Mutect2, MosaicHunter, or Strelka (Figures S1, S2, and S3E; details in Supplement 1) in terms of the sensitivity. We initially detected 23,554 somatic SNVs in brain tissue and 27,593 somatic SNVs in peripheral tissues as the raw call set. Because MuTect might show more false-positive calls owing to its higher sensitivity, we extensively excluded false-positive calls and artifacts mimicking low-level somatic mutations by adopting strict postfiltering processes, including high base quality scores, empirical Bayesian score, and manual inspection of sequenced reads (details in the Supplemental Methods in Supplement 1) (Figure 1A, B). As a result, we were able to identify 286 somatic SNVs in the brain and 340 in peripheral tissues (287 SNVs in liver tissue from 37 cases and 53 in heart and spleen tissues from 4 cases). To validate the identified somatic mutations

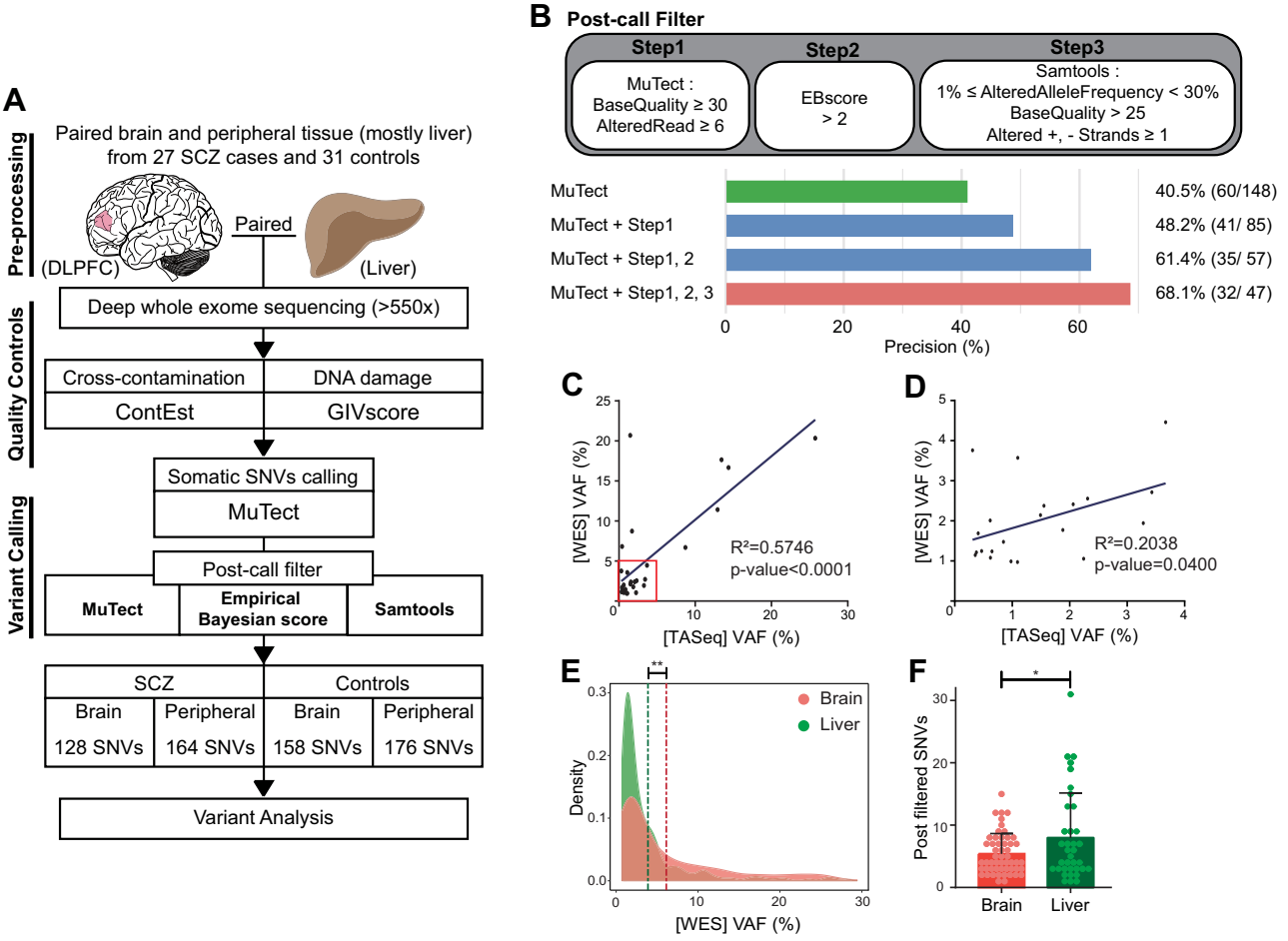

Figure 1. Detection of low-level somatic mutations in matched brain and peripheral tissues. **(A)** Schematic showing the analytic pipeline of low-level somatic mutations from 27 patients with SCZ and 31 control individuals. To improve the accuracy of variant calling, quality control measures were performed for BAM files to assess cross-individual contamination and damage that occurred during library preparation. With BAM files that passed quality control, MuTect was used to detect somatic mutations, followed by post–call filtering using empirical Bayesian score and SAMtools algorithms. **(B)** The post–call filter process increased the precision of detecting low-level somatic mutations. The three-step filtering increased the precision up to 68.1%. Candidate somatic SNVs were validated by TASeq and Sanger sequencing. **(C)** Concordance in VAFs between WES and TASeq. VAFs were highly correlated with each other (R^2 = .5746, $p <$ .0001). **(D)** Enlargement of red square in **(C)**: Concordance in VAFs of validated SNVs under 5% between WES and TASeq, where VAFs were highly correlated to each other (R^2 = .2038, p = .0400). **(E)** Distribution of VAFs for somatic SNVs in brain and liver tissues. **p = .0052. **(F)** Number of postfiltered SNVs in brain and liver tissues. *p = .0246. DLPFC, dorsolateral prefrontal cortex; EBscore, empirical Bayesian score; GIVscore, global imbalance value score; SCZ, schizophrenia; SNV, single nucleotide variation; TASeq, targeted amplicon sequencing; VAF, variant allele frequency; WES, whole exome sequencing.

from our strict pipeline, we randomly picked ~37% (47/128 SNVs) of filtered brain somatic SNVs from the brain tissue of individuals with SCZ and performed ultradeep TASeq (749,452.4×) and Sanger sequencing. If such a mutation was present as statistically reliable compared with previously estimated background errors, we considered using them as true calls (32). In the validation sequencing, we found that 68.1% of postfiltered SNVs were true calls (Figure 1B). Moreover, the VAFs of brain somatic SNVs found in deep WES were correlated with those in TASeq (Figure 1C, D). Next, we sought to investigate tissue-specific mutational profiles of somatic SNVs. To do so, we pooled all filtered somatic SNVs from brain and liver specimens (n = 286 and n = 287, respectively) and compared the distribution of VAFs and the average number of somatic SNVs between them. We found that the liver tissue showed significantly lower average VAFs (7.8% in brain and 5.5% in liver) and larger average numbers of somatic mutations (5.3 in brain and 7.8 in liver) (Figure 1E, F). Interestingly, the VAFs and number of liver somatic SNVs observed in our study were similar to those in previous reports using deep WES of liver tissue (33–36). These results suggest that deep WES followed by our strict filtering and analysis of somatic SNVs could exclude a substantial portion of false-positive calls and accurately detect low-level somatic mutations.

Mutational Profiles of Somatic Mutations in Patients With SCZ and Control Individuals

To examine differences in the profiles of somatic mutations between patients with SCZ and control individuals, we compared the number of somatic mutations, substitution type, and distribution of variant types in four groups: Control_Brain, SCZ_Brain, Control_Liver, and SCZ_Liver (Figure 2). Regarding the total number of somatic SNVs in patients with SCZ and control individuals, we found averages of 4.9 (average VAF = 8.0%) and 5.6 (average VAF = 7.6%) brain somatic SNVs, respectively, and 7.7 (average VAF = 5.5%) and 7.7 (average VAF = 5.6%) liver somatic SNVs, respectively (Figure 2A). There was no significant difference in the total number of somatic SNVs between the SCZ and control tissues. We also found that about 39.1% of the brain somatic SNVs in SCZ specimens and 33.5% in control specimens, and about 47.1% of the liver somatic SNVs in SCZ specimens and 41.5% in control specimens, were present in coding regions. Again, there was no significant difference in the distribution of substitution and variant types between the groups (Figure 2B, C). We then categorized somatic mutations as deleterious SNVs according to damage scores in PolyPhen, LRT, MutationTaster, MutationAssessor, CADD, and GERP and according to population-based allele frequencies (details in the Supplemental Methods in Supplement 1). We compared the number of deleterious SNVs between SCZ and control specimens and found no significant difference between the two groups (Figure 2D). Then, to explore the mutagenic sources of somatic mutations detected in brain and liver tissues, we observed mutation signatures of the somatic SNVs in the tissues using deconstructSigs (37). In both organs, signature 1 (known to be related to cytosine to thymidine deamination), which spontaneously occurs during cell proliferation, was found to be present in 61.8% of brain tissue and 59.8% of liver tissue (22) (Figure 2E, F). Overall, we were able to detect mutational signatures compatible with spontaneous accumulation of somatic mutations during cell proliferation in both the brain and liver. Furthermore, we explored the correlation between age and the proportion of C-to-T substitution that is prominent in both signatures and figured out that the proportion of SNVs with C-to-T substitution in the brain shows positive correlation with age and might arise from defective DNA mismatch repair (Figure S3H in Supplement 1) (38–40). Altogether, these results indicated that there are no quantitative differences in the mutation profiles (e.g., number, substitution type, distribution) of somatic SNVs between patients with SCZ and control individuals.

Brain Somatic Mutations Found in Patients With SCZ Are Enriched in Genes Associated With SCZ-Relevant Signaling Pathways

Next, we explored the involvement of the observed somatic mutations in the pathogenesis of SCZ. First, we examined mutation burden per exonic length between groups or between cases and controls to exclude gene length bias. There was no difference between groups or cases and controls (Figure S4A, B in Supplement 1). In addition, we compared the mutational burden per transcript length with deleterious mutations because deleterious mutations are in exons. As a result, deleterious mutation burden between groups was not different, and patients with SCZ did not have an increased mutational burden per transcript (Figure S4C, D in Supplement 1). Next, we performed gene length adjusted gene set enrichment tests with DNENRICH software (41). Specifically, we sought to extract significant pathway terms for patients with SCZ (for both brain and liver tissues) compared with control individuals or for SCZ_Brain compared with SCZ_Liver, Control_Brain, and Control_Liver. When we compared the genes with deleterious mutations, we found that in addition to dopaminergic synapse and neuroactive ligand–receptor interaction, glutamatergic synapse and long-term potentiation pathways were significantly enriched in patients with SCZ, particularly in brain tissue (Figure 3A and Figure S4F in Supplement 1).

We then sought to determine which specific genes would more likely be engaged in the pathogenesis of SCZ by examining literature-based evidence and protein–protein interactions. To do so, we estimated the protein–protein interactions of each protein putatively affected by the deleterious somatic mutations detected in the brain tissue from the patients with SCZ and control individuals. Remarkably, GRIN2B ranked as high as second, showing interactions with numerous proteins (Figure 3B). In addition, using the text-mining engine DigSee (42,43), we found that *GRIN2B* was the most frequently studied gene in SCZ among all 35 genes with deleterious somatic mutations identified from brain tissue from the patients with SCZ and control individuals (Figure 3B). Surprisingly, in our cohort, 2 patients were found to carry deleterious brain somatic mutations on *GRIN2B* (Table 1 and Table S3 in Supplement 2). Overall, these results indicated that the genes affected by the noted brain somatic mutations are associated with glutamate signaling pathways and that among them *GRIN2B* could be a target gene in the pathogenesis of SCZ.

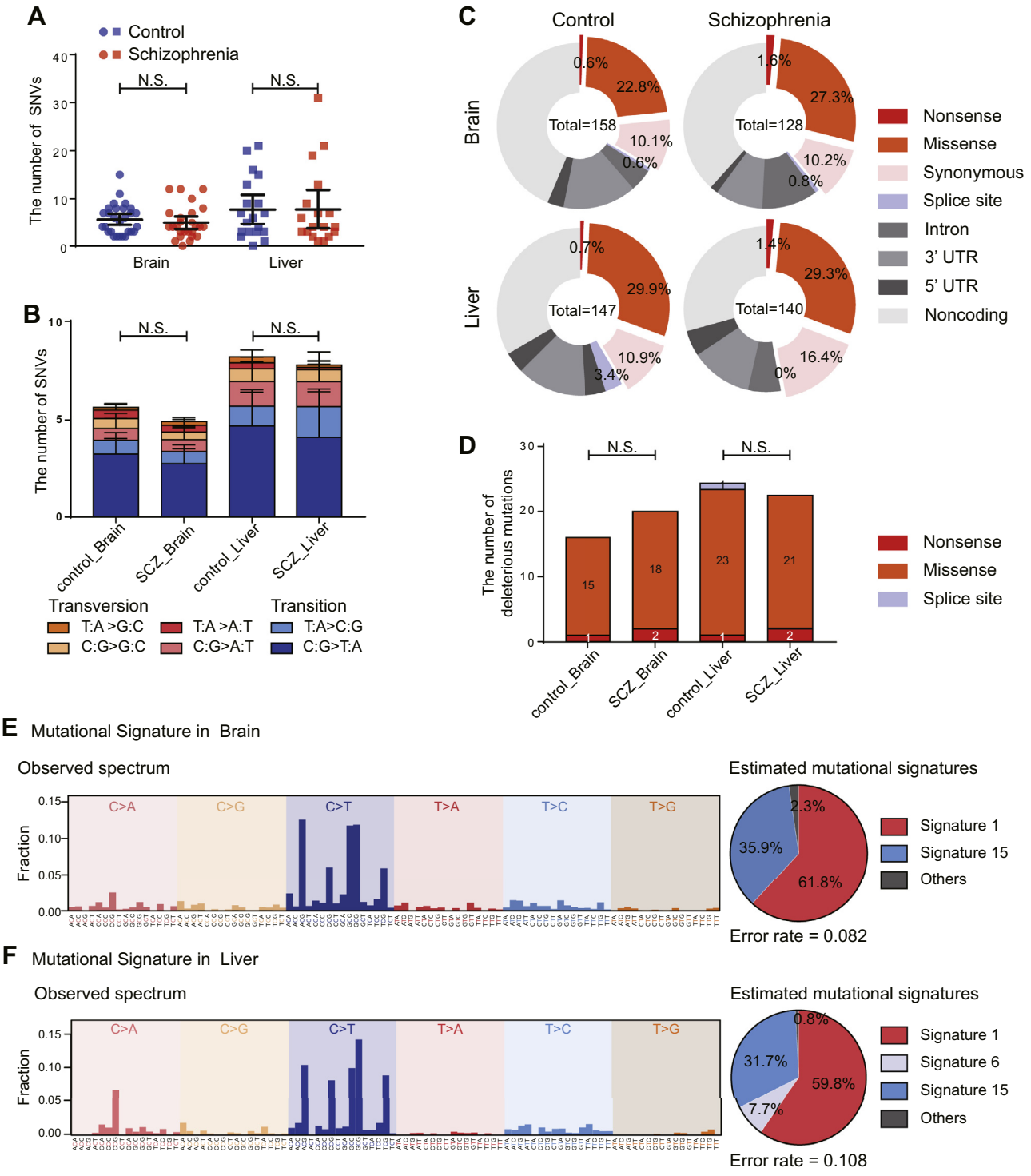

Figure 2. Mutational profiles of low-level somatic SNVs in patients with SCZ and control individuals. **(A)** Numbers of postfiltered SNVs in brain and liver tissues from patients with SCZ and control individuals. **(B)** Distribution of substitution types in brain and liver tissues from patients with SCZ and control individuals. **(C)** Distribution of variant types of SNVs in brain and liver tissues from patients with SCZ and control individuals. Among the called SNVs, about 40% of SNVs were in coding regions, and there was no significant difference in the distribution of variant types between groups. **(D)** Distribution of deleterious mutations in brain and liver tissues from patients with SCZ and control individuals. **(E, F)** Mutation signatures (COSMIC, exome adjusted) of somatic SNVs observed in brain tissue **(E)** and liver tissue **(F)**. Signature 1, known to be related to cytosine-to-thymidine deamination, which spontaneously occurs during cell proliferation, was found to be present in 61.8% of brain tissue and 59.8% of liver tissue. Error bars indicate 95% confidence intervals. The *p* values were calculated by one-way analysis of variance with Sidak's multiple comparison tests. N.S., nonsignificant; SCZ, schizophrenia; SNV, single nucleotide variation; UTR, untranslated region.

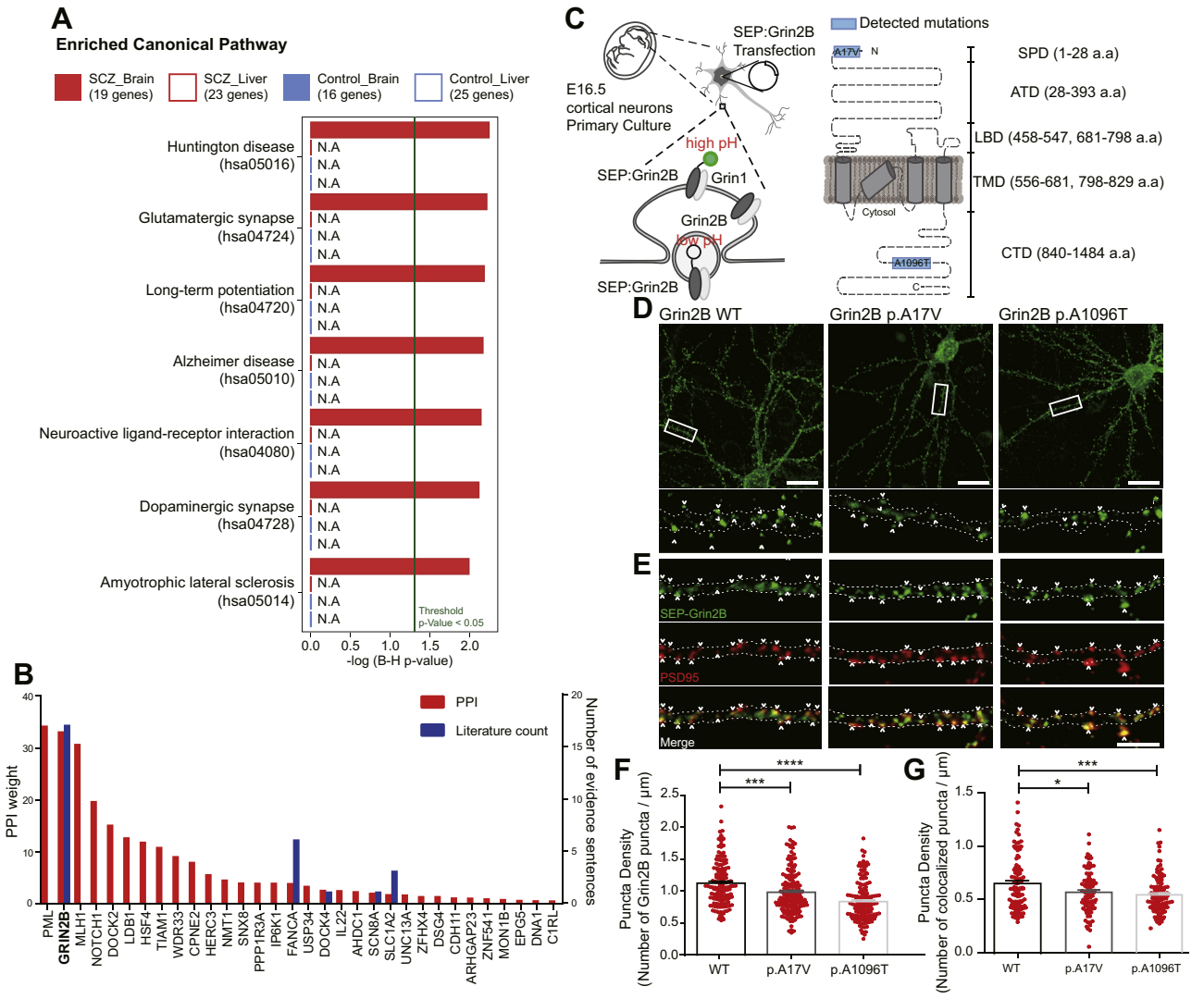

Figure 3. Genes with somatic single nucleotide variations found in brain tissue from patients with SCZ are associated with SCZ-relevant signaling pathways. **(A)** Enriched KEGG pathways of genes with deleterious mutations in the DNENRICH tool. The straight vertical line (green) running through the bars represents the threshold *p* value for the significant enrichment of a particular pathway. A multiple testing–corrected *p* value was calculated using the Benjamini–Hochberg method to control the rate of false discoveries in statistical hypothesis testing. **(B)** PPI weights and literature-based evidence of genes with deleterious somatic mutations found in SCZ brains. *GRIN2B* (bold) was ranked with high PPI weight and literature-based evidence. **(C)** The location of identified *GRIN2B* mutations and the experimental scheme for determining the effect of brain somatic mutations on the synaptic location of GRIN2B. SEP is a modified green fluorescent protein that fluoresces when it presents on the surface of cells. The positions of identified brain somatic mutations in *GRIN2B* are presented: signal peptide (c.C50T, p.A17V) and C-terminal domain (c.G3286A, p.A1096T). **(D)** Immunostaining for transfected SEP:GRIN2B in primary neuronal cultures at DIV14. Primary culture and immunostaining were repeated three times, and each experiment produced three coverslips for each group. Scale bars = 20 µm. **(E)** Immunostaining of SEP:GRIN2B colocalized with PSD95 in primary neuronal cultures at DIV14. Primary culture and immunostaining were repeated three times, and each experiment produced three coverslips for each group. Scale bars = 5 µm. **(F)** Bar graphs showing the puncta density of transfected GRIN2B per 1 µm of dendrite length in primary neuronal cultures at DIV14. WT: n = 19 neurons; p.A17V: n = 21 neurons; p.A1096T: n = 21 neurons. ***p = .0006, ****p < .0001. **(G)** Bar graphs displaying the density of SEP:GRIN2B puncta colocalized with PSD95 per 1 µm of dendrite length in primary neuronal cultures at DIV14. WT: n = 15 neurons; p.A17V: n = 12 neurons; p.A1096T: n = 15 neurons. *p = .0102, ***p = .0003. Error bars indicate SEM. The p values were calculated by one-way analysis of variance test with Sidak's multiple comparison tests. DIV, days in vitro; N.A, not available; PPI, protein–protein interaction; SCZ, schizophrenia; WT, wild-type.

Deleterious Brain Somatic Mutations in *GRIN2B* Can Disrupt the Synaptic Localization of Its Encoded Protein

In light of the above, we sought to investigate the detrimental impact of somatic SNVs on the function of GRIN2B in vitro. Among deleterious somatic SNVs found in *GRIN2B,* the sites of mutation were located on the signal peptide and C-terminal domains, both of which are important for the synaptic localization of GRIN2B (44,45) (Figure 3C). Based on this evidence, we hypothesized that brain somatic mutations in *GRIN2B* might disrupt GRIN2B localization on the surface of dendrites,

Table 1. Deleterious Mutations in Genes of Enriched Canonical Pathways in Patient Brain Tissue

Case	Gene	Mutation	VAF (%)	Pathway	
				Glutamatergic Synapse	HD and ALS
SCZ03	*GRIN2B*	NM_000834.3: c.50C>T (p.Ala17Val)	1.25	Risk allele leads to glutamate receptor hypofunction in patients with SCZ (73,74)	*GRIN2B* gene variation attribute age at onset in HD, utility as a biomarker for ALS (84,109)
SCZ08	*GRIN2B*	NM_000834.3: c.3286G>A (p.Ala1096Thr)	1.08		
SCZ05	*SLC1A2*	NM_004171.3: c.1682C>T (p.Ala561Val)	1.45	↓in the superior temporal gyrus and hippocampus of patients with SCZ (75)	Alterations in EAAT expression have been detected in epilepsy, HD, and ALS (110,111)
SCZ18	*DNAH1*	NM_015512.5: c.401C>A (p.Pro134Gln)	1.57	–	Identified as neurodegenerative disorder-relevant variant (112)

gnomAD_AF (%) was not available.

AF, allele frequency; ALS, amyotrophic lateral sclerosis; EAAT, excitatory amino acid transporter; HD, Huntington disease; SCZ, schizophrenia; VAF, variant allele frequency.

leading to SCZ symptoms through a defect in neuronal stability. To test this, we first examined whether the identified mutations affect the expression and localization of GRIN2B itself in human embryonic kidney 293T cells. We found that both of the brain somatic mutations decreased the surface expression of GRIN2B, not total amounts thereof (Figure S6B–E in Supplement 1). Furthermore, to observe defective synaptic localization of GRIN2B mutants in neurons, we transfected a plasmid construct, pCAG-SEP:GRIN2B, into primary cortical neurons isolated from embryonic day 16.5 mice and performed immunofluorescence staining after 14 days in vitro with SEP, a green fluorescent protein–based super-ecliptic pHluorin that fluoresces green when located on or outside of the cell membrane, not inside of cellular vesicles (46) (Figure 3C). Interestingly, we observed significantly decreased GRIN2B puncta density in both GRIN2B mutant groups, suggesting that brain somatic mutations found in *GRIN2B* cause defective dendritic surface localization (Figure 3D, F). In addition, we observed significant decreases in the density of GRIN2B puncta colocalized with PSD95, thereby showing that GRIN2B mutants cannot properly localize on postsynapses (Figure 3E, G). Meanwhile, one study showed that mutations in the C-terminal domain of *GRIN2B* from patients with autism decreased dendritic spine density (47). Thus, we also checked PSD95 puncta density in dendrites of cultured neurons and found that the density of PSD95 puncta was significantly decreased, suggesting the disruption of dendritic spine morphology in GRIN2B p.A1096T-expressing neurons (Figure S6A in Supplement 1). Taken together, these results indicated that brain somatic mutations in *GRIN2B* disrupt the localization of its encoded protein to dendrites and hinder proper synapse formation.

Deleterious Germline, Mosaicism, and Brain Somatic Mutations in SCZ-Risk Genes Contribute to SCZ

We tried to elucidate the genetic architecture of SCZ, which might be categorized into germline, mosaicism, and brain somatic mutations, according to differential time points of the mutations (Figure 4). Throughout this study, we observed that 15.4% (4/26) of patients with SCZ carried deleterious brain somatic mutations in known SCZ-risk genes, including *GRIN2B, SLC1A2,* and *DNAH1,* whereas none of the control individuals did (Table 1 and Figure S5D in Supplement 1). Using Fisher's exact test for comparison, we have confidence that deleterious brain somatic mutations in known SCZ-risk genes had a significant effect on our cohort ($p = .0473$).

In addition to the brain somatic mutations, however, germline mutations and mosaicism might also contribute to the development of SCZ. To discover germline mutations that possibly contribute to the aberrant genetic structure of SCZ, we used HaplotypeCaller (48) and called germline single mutations from our dataset. We then adopted a previously published genome-wide association study dataset covering SCZ-risk genes (9). As a result, we found that 11.5% (3/26) of the patients with SCZ carried rare deleterious germline mutations in known SCZ-risk genes. Interestingly, however, we discovered that 21.4% (6/28) of the control individuals exhibited deleterious mutations in the same SCZ-risk genes. Through Fisher's exact tests, we found that the frequency of deleterious germline mutations did not affect our cohort.

To identify mosaicism, we also examined whether patients with SCZ carried deleterious mosaicism shared between brain and peripheral (liver, spleen, or heart) tissues in SCZ-risk genes. To do so, we performed Mutect2 (49) and called mutations shared in both regions and compared the average number and the average VAFs of mosaicism SNVs between patients with SCZ and control individuals. We found that the two groups had similar average counts (0.6 and 1, respectively) and VAFs (5.9% and 6.2%, respectively) (Figure S5A, B in Supplement 1) and that brain and peripheral tissues did not have differences in VAFs (6.2% and 6.0%, respectively) (Figure S5C in Supplement 1). Furthermore, we extracted SCZ-risk genes with rare deleterious mosaicisms (CADD $>$ 20, gnomAD $<$ 0.01%) from our cohort. We then compared previously published exome sequencing-datasets covering SCZ-risk genes (50,51) and found that 3.8% (1/26) of the cases carried rare deleterious mosaicisms, although no gene overlapped with the genome-wide association study dataset (9). We compared the frequency of deleterious mosaicisms in patients with SCZ and control individuals by Fisher's exact test, and there was no significant difference, possibly owing to the small size of our cohort. Taken together, our results

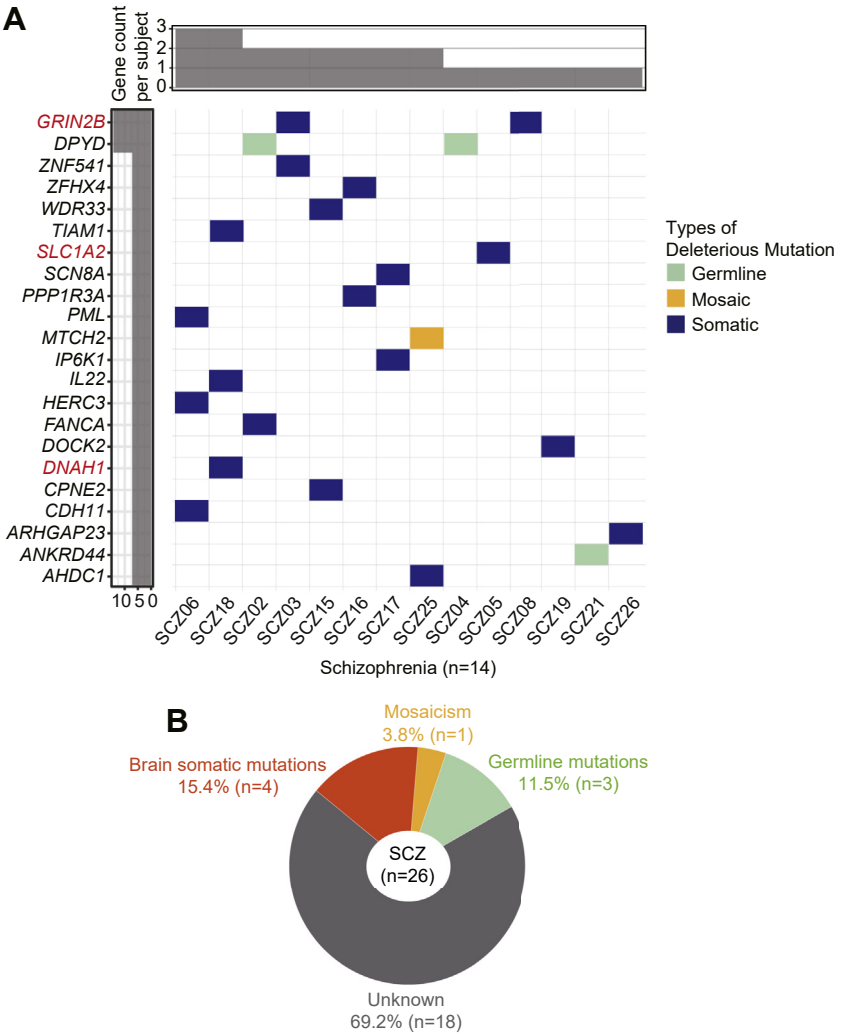

Figure 4. Deleterious brain somatic mutations contributing to the genetic architecture of SCZ. **(A)** Counts and names of mutated genes per patient with SCZ carrying deleterious brain somatic mutations, mosaicisms, and/or germline mutations in known SCZ-risk genes. Letters in red indicate genes significantly enriched in SCZ-relevant signaling pathways (Table 1). **(B)** Proportions of patients with SCZ with a deleterious germline, mosaicism, or brain somatic mutation in known SCZ-risk genes or genes enriched for canonical SCZ-relevant signaling pathways. As shown, 11.5% (3/26) of patients with SCZ carried deleterious germline mutations, 3.8% (1/26) carried mosaicisms, and 15.4% (4/26) carried deleterious brain somatic mutations. SCZ, schizophrenia.

suggest that deleterious brain somatic mutations in SCZ-risk genes might contribute significantly to SCZ (50).

DISCUSSION

In this study, we outlined the ways in which low-level somatic mutations may contribute to the pathogenesis of SCZ by performing deep WES on brain and matched liver (or spleen and heart) tissues from 27 patients with SCZ and 31 control individuals, followed by strict validation sequencing and comprehensive bioinformatic analysis. As a result, we were able to identify 286 somatic SNVs in the brain and 340 in peripheral tissues. Interestingly, there were no quantitative differences in the mutation profiles (e.g., number, substitution type, distribution) of somatic SNVs between patients with SCZ and control individuals. However, we found that deleterious somatic mutations detected in the brains of patients with SCZ were enriched in SCZ-related pathways, including glutamatergic synapse, dopaminergic synapse, and long-term potentiation pathways. In addition, we discovered that brain somatic mutations in *GRIN2B* disrupted the localization of GRIN2B to the surface of dendrites in cultured primary neurons.

The pathways that were enriched with the deleterious somatic mutations have previously been reported to be related to SCZ. Previous genetic studies have reported the positive genetic correlation of Huntington disease, AD, and amyotrophic lateral sclerosis disease with SCZ (52–55). In AD, Soheili-Nezhad *et al.* uncovered that genes related to synaptic regulation, such as synaptogenesis and glutamate receptor signaling pathways, are dysregulated somatically in affected brain regions, the hippocampus and temporal cortex (56). In addition, similar low-level brain somatic mutations have been discovered in hippocampal formations of AD brains, where neurofibrillary tangles initiate, and have been associated with dysregulation of tau phosphorylation (22). SCZ shares similar pathophysiological features with AD, including regional brain dysfunction and synaptic deficits in affected regions. Previous research showed dysregulation of autophagy only in the affected brain region—the hippocampus— and not in blood

(57). Furthermore, disruption of long-term potentiation and neuroactive ligand–receptor interaction have been implicated in SCZ (58–62). In addition, abnormal regulation of dopamine has been found to induce the symptoms of SCZ (63,64). In line with our results, numerous studies have shown that the glutamatergic synapse pathway is directly involved in the pathogenesis of SCZ (65,66); NMDA receptors sensing glutamate regulate excitatory neurotransmission and neuronal development and plasticity (67), and impairment thereof has been found to induce symptoms of SCZ (68–71). Furthermore, disruption of GRIN2B in glutamatergic synapses can lead to an imbalance between excitation and inhibition, thereby eliciting psychiatric symptoms (72).

Interestingly, 3 patients with SCZ carried deleterious mutations in genes encoding proteins involved in glutamate receptor signaling (73–77). These mutations were *SLC1A2* p.A561V (in case SCZ05), *GRIN2B* p.A17V (in case SCZ03), and p.A1096T (in case SCZ08), which have never been reported in the general population (e.g., gnomAD). The *SLC1A2* gene encodes EAAT2, which plays a role in clearing glutamate from synapses. An association between genetic variation in *SLC1A2* and SCZ has been suggested in several studies (78,79). Meanwhile, *GRIN2B* encodes a subunit of the NMDA receptor, which plays a key role in the postsynapse. Researchers have shown that disruption of GRIN2B induces an imbalance in excitatory/inhibitory synaptic drive (80). GRIN2B has been reported to be associated with several psychiatric diseases, including SCZ, autism, and intellectual disorder, and with other neurological diseases such as epilepsy (24,47,81–88). General disease-causing mutations associated with early-onset intellectual disability and developmental/neurological disorders accompanied by epilepsy are detected as germline mutations or mosaicism with high frequency, and most of them are in ligand-binding and transmembrane domains (89,90). Disruptive mutations in these domains alter GRIN2B function such as ligand binding or ion channel properties (91,92). On the other hand, most psychiatric disorder–related mutations, especially SCZ-associated ones, induce mislocalization of GRIN2B, and these mutations are located on the C-terminal domain (47,93). The N-terminal and C-terminal domains of GRIN2B contain posttranslational modifications, such as phosphorylation, and protein-interacting sites that are crucial for receptor trafficking and localization (67). Deleterious mutations in these domains might interrupt the interaction with other proteins and can alter GRIN2B localization. Mutations in *GRIN2B* are also known to be associated with SCZ (82,83,88). In addition, many studies have shown that dysregulation of surface distributions of GRIN2B-containing NMDA receptors can induce defects in neuronal stability, plasticity, and spine density (44,47,67,94), which are important hallmarks in the etiology of several cognitive and psychiatric disorders (47,95–101). In this study, we showed that brain somatic brain mutations in *GRIN2B* are in N-terminal and C-terminal domains and lead to reduced expression of GRIN2B along dendrites. These brain somatic mutations in GRIN2B might alter the function of a subset of neurons with mutations and related circuits. To our knowledge, this is the first evidence to indicate that brain somatic mutations in SCZ have a deleterious functional impact on glutamate receptors.

Deep WES in matched brain–peripheral tissues allowed us to detect brain somatic SNVs at low-level VAFs, with 59.8% (171/286) of the detected brain somatic variants being present at VAFs lower than 5%. The deleterious somatic mutations in *GRIN2B* presented in SCZ brains with VAFs as low as 1.25% (in case SCZ03) and 1.08% (in case SCZ08). Previous studies have shown that a subset of neurons with a disrupted gene in the focal cortical region is sufficient to cause dysfunction of the entire brain, thereby leading to neuropsychiatric symptoms or related disorders (20,23–25,27,28,102–105); for example, focal disruption of Arp2/3 in the frontal cortex leads to psychiatric symptoms, such as abnormal locomotor behavior, by disturbing cortical-to-midbrain circuits (106). Somatic activating mutations in mTOR pathway genes with VAFs ∼1% in the focal cortical region cause spontaneous behavioral seizures in mice (19). In addition, low-level BRAF V600E somatic mutations arising during brain development cause intractable epilepsy (107). Loss of function somatic mutations in *SLC35A2,* which encode UDP-galactose transporter in Golgi complex, is associated with focal epilepsy (21,26). Focal deletion of NaV1.1 sodium channel in GABAergic (gamma-aminobutyric acidergic) neurons is sufficient to cause epilepsy (108). Consistent with our study, these previous studies showed the possibility that focal neuronal changes in synaptic structure, channel conductivity, or electrophysiological activity elicited by low-level brain somatic mutations can alter neuronal circuits and lead to neuropsychiatric disorders.

In line with this idea, it could be possible that our newly detected brain somatic mutations on *GRIN2B* might induce psychiatric symptoms through disruption of GRIN2B localization in the focal area of the DLPFC and related neural networks. This will be an interesting area of future research. Overall, our study suggests the importance of low-level deleterious brain somatic mutations into the molecular genetic architecture of SCZ and potentially other psychiatric diseases.

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M-HK, ShK, and JHL conceived the study and organized experiments. M-HK, MJW, and ShK prepped human tissue samples and performed WES. M-HK, IBK, JL, DHC, and YA performed genetic studies and bioinformatic analysis. M-HK, JL, JHK, JSP, and SyK established postfiltering processes. M-HK and SyK performed validation sequencing. M-HK, SMP, RK, and KK performed in vitro functional studies with primary neurons and cell lines. M-HK, IBK, DHC, SMP, and JHL wrote the manuscript with input from all authors.

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