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Rare variants and de novo variants in mesial temporal lobe epilepsy with hippocampal sclerosis

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Abstract

Objective

We investigated the role of rare genetic variants and of de novo variants in the pathogenesis of mesial temporal lobe epilepsy related to hippocampal sclerosis (MTLE-HS).

Methods

Whole-exome sequencing (WES) was performed in patients with MTLE-HS and their unaffected parents (trios). Genes or gene sets that were enriched with predicted damaging rare variants in the patients as compared to population controls were identified. Patients and their parents were compared to identify whether the variants were de novo or inherited.

Results

After quality control, WES data from 47 patients (26 female), including 23 complete trios, were available for analysis. Compared with population controls, significant enrichment of rare variants was observed in *SEC24B*. Integration of gene set data describing neuronal functions and psychiatric disorders showed enrichment signal on fragile X mental retardation protein (FMRP) targets. Twenty-one de novo variants were identified, with many known to cause neuropsychiatric disorders. The FMRP-targeted genes also carried more de novo variants. Inherited compound heterozygous and homozygous variants were identified.

Conclusions

The genetic architecture underlying MTHE-HS is complex. Multiple genes carrying de novo variants and rare variants among FMRP targets were identified, suggesting a pathogenic role. MTLE-HS and other neuropsychiatric disorders may have shared biology.

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Glossary

ASD = autism spectrum disorder; **ExAC** = Exome Aggregation Consortium; **FMRP** = fragile X mental retardation protein; **GWAS** = genome-wide association study; **HS** = hippocampal sclerosis; **IL** = interleukin; **MAF** = minor allele frequency; **MGI** = Mouse Genome Informatics; **mGluR** = metabotropic glutamate receptor; **MTLE-HS** = mesial temporal lobe epilepsy related to hippocampal sclerosis; **NMDAR** = NMDA receptor; **SNP** = single nucleotide polymorphism; **WES** = whole-exome sequencing.

Mesial temporal lobe epilepsy related to hippocampal sclerosis (MTLE-HS) is among the most drug-resistant types of focal epilepsy.[1] Histologically, MTLE-HS is characterized by atrophy and astrogliosis of the amygdala, hippocampus, parahippocampal gyrus, and the entorhinal cortex.[2] In drug-resistant patients, resection of the sclerotic hippocampus and the surrounding mesial temporal structures can be an effective treatment[3]; hence, MTLE-HS remains one of the most common indications for epilepsy surgery.[4] Understanding the molecular basis of MTLE-HS may lead to identification of novel drug targets and alleviate the need for invasive treatment.

The pathogenesis of MTLE-HS is unknown. Several studies have investigated the role of common susceptibility variants in the pathogenesis of MTLE-HS.[5] Early studies reported associations between single nucleotide polymorphisms (SNPs) in interleukin *(IL)-1, PDYN, GABBR1,* and *PRNP* and mesial temporal lobe epilepsy, but the results have been controversial.[6] A recent genome-wide association study (GWAS) identified an association with *SCN1A* polymorphisms.[7] However, a large heritability study of focal epilepsy suggested that common variants explained only 3% of heritability.[8]

Recently, whole-exome sequencing (WES) of 356 trios discovered 429 de novo variants in patients with epileptic encephalopathies, with recurrent mutations in 19 genes.[5] We hypothesized that, similar to other focal epilepsy syndromes,[9] both rare and de novo variants may underlie the unexplained genetic susceptibility to MTLE-HS. WES was applied in a recent study of patients with a variety of common generalized and focal epilepsy syndromes,[10] but trios were not examined. We performed WES on patients with MTLE-HS and some unaffected parents in this study.

Methods

Study design

We performed WES on patients with MTLE-HS and their unaffected parents. This enabled us to examine the effects of rare variants among all the patients by comparing with population controls and to identify genes containing de novo and inherited variants in trio-based analysis.

Participants

Patients with MTLE-HS (probands) and their parents were recruited from 3 regional hospitals (Prince of Wales Hospital, United Christian Hospital, and Queen Elizabeth Hospital) in Hong Kong. Inclusion criteria of probands were "pure" MTLE-HS with concordant findings from seizure semiology, EEG (interictal and ictal recording during prolonged video EEG monitoring), MRI (1.5T or 3T) findings characteristic of hippocampal sclerosis (HS), and histologic confirmation of HS in patients who had undergone resective epilepsy surgery.[11] All patients were ethnic Han Chinese with an age at onset of epilepsy of ≥2 years. Patients were excluded if there was evidence of extratemporal lobe seizures, they had no history of seizure, had psychogenic nonepileptic seizures, or had other epileptogenic lesions identified on MRI. Parents of probands were eligible for inclusion if they did not have epilepsy or history of febrile seizure. Each participant provided either venous blood or saliva samples from which DNA was extracted for sequencing using standard protocols. A total of 48 patients with MTLE-HS were enrolled. Both parents were recruited for 23 patients, forming complete trios. The study was approved by the ethics committees of the participating hospitals. All participants or their legal guardians provided written informed consent.

Population controls

For the purpose of quality assessment and association testing, WES data from 692 Hong Kong Han Chinese participants (298 men and 394 women) were added to the calling set (Supplemental materials, links.lww.com/NXG/A55) (mean age: 41.1 years, range: 15–55 years). They were participants in a population-based study investigating lumbar disc degeneration and did not have a history of developmental or neuropsychiatric disorders.[12]

Standard protocol approvals, registrations, and patient consents

The study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (ref. No. 2004.068 and 2004.268). All participants or their legal guardians provided written informed consent.

Whole-exome sequencing and bioinformatics pipeline

Detailed methodology for WES and the bioinformatics pipeline including quality control, variant calling, and in silico analysis, is provided in supplementary materials.

Association analysis

Gene-based association tests

Case-control analyses of rare variants (minor allele frequency [MAF] <1% in the 1000 Genomes Project Phase III, Exome Aggregation Consortium (ExAC), and dbSNP137 databases) included all the patients with MTLE-HS and population

controls. Principle component analysis on the patient group (N = 47) and control group (N = 692) revealed no systematic bias between the 2 data sets (figure e-1, links.lww.com/NXG/A55). Burden tests on rare damaging variants were performed per gene from the human RefGene database. Three criteria were adopted to select rare damaging variants within genes for the test: (1) single nucleotide variants with MAF >1% in the 1000 Genomes Project Phase III, ExAC, and dbSNP137 databases were excluded; (2) missense and nonsense variants that passed quality control were used in the tests; and (3) only genes carrying 3 or more variants were included in the gene-based association tests. A burden-style test from PLINK/SEQ (https://atgu.mgh.harvard.edu/plinkseq) was chosen for the purpose. The burden test was performed using 1,000,000 rounds of adaptive permutations. Multiple testing corrections were applied for the number of tested genes using the false discovery rate.[13] Gene annotation and damaging variant prediction was performed using KGGSeq, which combines multiple prediction methods.[14]

Set-based association tests

For set-based association tests, we considered groups of genes with similar biological functions as the unit of testing (gene set). We first included a set of focal epilepsy candidate genes[15] (table e-1, links.lww.com/NXG/A55). In addition, given the high prevalence of psychiatric disorders among patients with focal epilepsy,[16] we curated additional gene sets by considering those of psychiatric disorders and of important neuronal functions (table e-2, links.lww.com/NXG/A55). Gene sets were obtained from the "Genebook" website(atgu.mgh.harvard.edu/~spurcell/genebook/genebook.cgi) previously used for a large-scale exome sequencing study of schizophrenia.[17] The primary and secondary gene sets on the website cover 2,546 gene candidates involved in important systems of neuronal functions and previous studies of psychiatric disorders, including intellectual disability, schizophrenia, fragile X syndrome, and autism spectrum disorders (ASD). In particular, rare variants of fragile X mental retardation protein (FMRP)-targeted genes have been found to be enriched in multiple psychiatric disorders (fragile X, ASD, and schizophrenia).[18] FMRP is encoded by the gene *FMR1*, an RNA-binding protein that regulates translation of synaptic genes for normal neurogenesis.[19] We combined the 2 previously reported FMRP sets[20,21] to better evaluate the importance of FMRP targets in MTLE-HS. The same criteria for variant selection and statistical tests as used for gene-based association were used for gene set–based association.

Trio-based analysis

To identify de novo and inherited variants attributed to MTLE-HS, trio-based analyses were performed in the subgroup of MTLE-HS patients with complete trios.

De novo variants and gene set enrichment analysis

KGGSeq was used for the discovery and annotation of de novo variants in the trios. The predicted damaging effects and variation intolerance scores were annotated per gene and per variant. In addition to the quality control described above, each de novo variant required a read depth ≥8 to be called. Mutations found in any of the 3 population databases (dbSNP137, ExAC, or 1000 Genome phase III) were excluded. All de novo variants were validated by Sanger sequencing. The nonsynonymous to synonymous ratio was compared with other exome sequencing studies. The candidate focal epilepsy gene list (table e-1, links.lww.com/NXG/A55) has been compared with our de novo variant gene list for overlaps. The 25 gene sets tested for rare variant association were also tested for enrichment of de novo variants by hypergeometric test, so as to assess whether the same biological network was disrupted among the de novo genes.

Inherited variant analysis: homozygotes and compound heterozygotes

To investigate the effect of heterozygous and homozygous (double hit) events, genes carrying ≥2 mutations in heterozygous and homozygous configurations were summarized. At least 1 hit inherited from each unaffected parent is required to fit the sporadic nature of the recruited samples. Rare variants at MAF ≤ 1% (in the 1000 Genomes Project Phase III, ExAC, and dbSNP137 databases) were considered in the analysis. We considered the following scenarios of genes carrying double hit variants, which are rare/absent in the population: (1) candidate genes suggested by functional databases (Phenolyzer and SynaptomeDB) and with adjusted p values <0.05; (2) both contributing variants are nonsense and not found among phased controls (Genome of the Netherlands project suggested double knockout by Loss-of-Function variants are very rare)[22]; and/or (3) genes with de novo variants carried by the patients with MTLE-HS. Details of each approach are described in Supplemental materials, links.lww.com/NXG/A55. The recurrences in the subsequent gene list were further investigated for their relevance to MTLE-HS.

Integrative genomic annotation

To investigate whether the gene sets suggested by the association tests are enriched at the transcriptome level, we reviewed published expression studies on hippocampal tissues of patients with MTLE-HS to derive an intersected list of dysregulated genes (table e-3, links.lww.com/NXG/A55). Seven expression studies were identified after excluding studies on other types of epilepsies. Genes reported by at least 2 studies were included in the gene list. The genes were tested for enrichment with the 25 candidate gene sets derived from the "Genebook" website. Hypergeometric tests were performed, where p values were corrected for the number of tests (25 sets × 7 studies).

To investigate the functional relevance of the candidate genes carrying de novo variants, we queried the gene list against Mouse Genome Informatics (MGI) and ClinVar.[23] Genes reported to cause any neural abnormalities in knockout mouse models or by human genetic studies were tabulated.

Results

Participants

A total of 48 patients with MTLE-HS were enrolled. Sequence data from 1 patient did not pass quality control and was excluded (supplemental data, links.lww.com/NXG/A55), leaving 47 patients (26 female) for further analysis. The characteristics of the patients are shown in table 1. The median age at onset of epilepsy was 17.7 years (range 1.5–43 years), and the median age of recruitment was 39.5 years (range 13–57.4 years). There were equal numbers of patients with left and right HS (21 patients each); 5 patients had bilateral disease. Thirty patients with unilateral drug-resistant MTLE-HS had undergone epilepsy surgery (anterior temporal lobectomy and amygdalohippocampectomy). Seizure onset was confirmed on ictal video EEG recording in all patients before surgery. Histology confirmed HS in the resected hippocampus in all patients. Nineteen were seizure-free after surgery. Both parents were recruited for 23 patients, forming complete trios. None of the parents had a history of epilepsy or febrile seizure. All probands and parents were of Han Chinese descent.

Association analysis

Gene-based and set-based association tests

Gene-based p values are shown in table 2. The *SEC24B* gene remained significant after correction for multiple testing ($q < 0.041$, 5,154 genes). The association was contributed by 4 predicted damaging variants, 3 of which were found in the patients and 1 in the population controls.

Table 3 shows results of the set-based tests using 25 gene sets. After correction for multiple comparisons, significant enrichment was observed in the FMRP-related gene sets. The bigger FMRP set[20] achieved a higher significance level, including all rare variants in the comparison ($p < 3.88 \times 10^{-4}$). When restricting to the rare predicted damaging variants, the unified set of 2 FMRP gene sets[20,21] also achieved statistical significance ($p < 2.89 \times 10^{-4}$). There was no significant enrichment for rare variants in the candidate focal epilepsy gene set.

Trio-based analysis

De novo variants

In total, 27 de novo variants were identified. One variant was excluded from validation because of technical difficulties. Among the remaining 26 variants, 21 were validated by Sanger sequencing. Therefore, our analysis pipeline achieved a true positive rate of 81% (21/26). There were no recurrent de novo mutated genes. The validated de novo mutations were found in 13 patients (8 had 1, 2 had 2, and 3 had 3 mutations). Of the 21 validated de novo mutations, 18 were nonsynonymous and 3 were synonymous (table 4). The nonsynonymous to synonymous variant ratio of all de novo variants was 6:1 ($p < 0.22$), which is higher than the neutral rate reported in other studies (2.8:1)[24] and is higher than that reported in patients with other neuropsychiatric disorders, including intellectual disability (5.6:1), ASD (3.1:1), and schizophrenia (5.1:1)[24] (table e-4, links.lww.com/NXG/A55). Two of the patients with de novo variants have a family history of epilepsy, implying that, as expected, not all of the de novo variants cause epilepsy.

The list of the 18 genes carrying the nonsynonymous de novo variants was compared with 3 studies investigating de novo variants in different neuropsychiatric disorders (epileptic encephalopathies,[25] ASD,[26] and schizophrenia[24]). We found that 5 of 18 genes were reported by one of the studies, and 3 genes (*ROBO4, NLGN3,* and *CEP170B*) were found to overlap with the ASD study (table 4). However, none of the 18 genes affected by nonsynonymous de novo variants was found in the focal epilepsy gene set (table e-1, links.lww.com/NXG/A55).

Gene set enrichment analysis for de novo variants

The enrichment test suggests that the FMRP-targeted genes also carried more de novo variants in the patients with MTLE-HS, which is in agreement with the association results mentioned above (table e-5, links.lww.com/NXG/A55). Although testing of the 2 FMRP-targeted gene sets[20,21] separately were not significant, the merged FMRP set achieved a significant p value ($p < 0.0013$).

Inherited variants: homozygotes and compound heterozygotes

We assessed the patients for inherited damaging variants acting in a recessive manner, either as compound heterozygotes or homozygotes. Genes fulfilling such criteria were considered to be carrying "double-hit" variants. For loss-of-function variants, we considered all rare events (MAF < 1%). Only 1 gene, *P2RX7*, was identified, harboring a homozygous nonsense variant (NM_002562:c.1591G>T:p.E531*) in a single patient. The mutation was validated by Sanger sequencing.

For missense variants, we considered genes carrying double hit variants, which are recurrent in MTLE-HS or present in the de novo gene list. In addition to restricting MAF to 1% in population databases, we used 3 criteria described in the Methods section to identify 3 genes carrying missense variants as either homozygous or compound heterozygous: *CEP170B*, *UBR4*, and *CALHM1*.

We found 2 patients carrying homozygous or compound heterozygous rare variants in *CEP170B.* Among 4 of the contributed variants, one of them is a de novo variant. The 2 compound heterozygous variants were validated. One of the probands carrying a compound heterozygous variant in *CEP170B* had a reported family history of epilepsy (sibling and son) (table e-6, links.lww.com/NXG/A55).

Integrative genomic annotation

Comparison between the 25 gene sets used in the association tests and published transcriptomic studies of MTLE-HS

Table 1 Clinical characteristics of patients with mesial temporal lobe epilepsy related to hippocampal sclerosis and their corresponding genes reported

Trios group (included in association analysis and trio-based analysis)											
Participant no.	**Sex**	**Age at recruitment**	**Age at onset**	**Duration of epilepsy**	**History of FS[1]**	**Epilepsy surgery**	**Surgical outcome[a]**	**Lateralization**	**Family history of epilepsy or FS**	**Inheritance model[b]**	**Gene name**
5	Female	34.3	6	28.3	Yes	No	—	L	Paternal granduncles (epilepsy)	—	—
8	Male	57.4	29	28.4	No	No	—	L	N	—	—
9	Female	52.8	34	18.8	No	No	—	R	N	De novo	*FAM65A*
										CH	*CALHM1*
										Rare burden	*SEC24B*
12	Male	42	13	29	No	No	—	B	N	De novo	*CEP170B*
										De novo	*FGB*
										De novo	*MASTL*
15	Female	25.5	12	13.5	No	Yes	P	L	N	Homozygous	*P2RX7*
17	Male	39.7	27	12.7	No	Yes	G	R	N	—	
19	Male	50.3	14	36.3	Yes	No	—	L	N	De novo	*GRASP*
										De novo	*SLC5A12*
										De novo	*CDC42EP1*
21	Female	40.9	17	23.9	No	No	—	B	N	—	—
22	Female	33.7	17	16.7	Yes	Yes	G	R	N	—	—
24	Male	31.1	23	7.9	Yes	No	—	L	N	De novo	*NBEAL1*
										CH	*UBR4*
25	Male	35.2	31	3.7	No	Yes	G	R	N	Homozygous	*CEP170B*
26	Female	25.6	2.3	23.3	Yes	Yes	P	L	N	De novo	*NPC1L1*
										Rare burden	*SEC24B*
28	Female	21.9	13	8.6	No	Yes	G	R	N	—	—
30	Female	41.1	12	29.3	Yes	Yes	G	B	N	De novo	*ANXA6*
33	Male	17.6	6	11.3	No	Yes	G	R	N	CH	*CALHM1*
34	Female	31.3	17	14.4	No	Yes	G	L	N	De novo	*NLGN3*

Continued

Table 1 Clinical characteristics of patients with mesial temporal lobe epilepsy related to hippocampal sclerosis and their corresponding genes reported *(continued)*

Trios group (included in association analysis and trio-based analysis)

Participant no.	Sex	Age at recruitment	Age at onset	Duration of epilepsy	History of FS[1]	Epilepsy surgery	Surgical outcome[a]	Lateralization	Family history of epilepsy or FS	Inheritance model[b]	Gene name
37	Female	31.5	15	16.6	Yes	No	—	R	N	De novo	*SBSPON*
39	Female	46.4	7	39.6	Yes	No	—	L	N	De novo	*BAIAP2*
										De novo	*RASEF*
40	Female	47	36	10.9	No	No	—	R	Elder brother (epilepsy) and son (febrile convulsion)	De novo	*ROBO4*
										CH	*CEP170B*
41	Male	25.9	6	19.8	Yes	No	—	L	Elder sister (febrile convulsion)	—	—
43	Male	53.6	10	43.8	Yes	No	—	R	Younger sister (epilepsy)	De novo	*PLEC*
										De novo	*TACC2*
44	Male	42.9	14	28.9	No	No	—	R	N	De novo	*BHLHE40*
45	Male	49.3	21	27.9	No	No	—	L	N	—	—

Cases-only group (included in association analysis)

Participant no.	Sex	Age at recruitment	Age at onset	Duration of epilepsy	History of FS	Epilepsy surgery	Surgical outcome[a]	Lateralization	Family history of epilepsy or FS	Inheritance model[b]	Gene name
1	Female	42.4	29	13.4	No	Yes	P	B	N	—	—
2	Female	25.7	19	6.7	No	Yes	G	R	N	—	—
3	Female	56.2	43	13.2	No	Yes	P	R	Younger sister (epilepsy)	—	—
4	Female	38.7	9	29.7	Yes	Yes	P	R	N	—	—
6	Female	35.1	6	29.1	No	Yes	G	L	N	—	—
7	Female	49	6	43	No	Yes	G	L	N	—	—
10	Female	52.2	30	22.2	No	No	—	L	N	—	—
11	Female	39.5	10	29.5	Yes	Yes	G	R	N	—	—
13	Male	46.3	33	13.3	No	Yes	P	L	N	—	—

Continued

Table 1 Clinical characteristics of patients with mesial temporal lobe epilepsy related to hippocampal sclerosis and their corresponding genes reported *(continued)*

Cases-only group (included in association analysis)											
Participant no.	**Sex**	**Age at recruitment**	**Age at onset**	**Duration of epilepsy**	**History of FS**	**Epilepsy surgery**	**Surgical outcome[a]**	**Lateralization**	**Family history of epilepsy or FS**	**Inheritance model[b]**	**Gene name**
14	Male	42.7	16	26.7	Yes	Yes	G	L	N	—	—
16	Male	33	17	16	No	Yes	G	R	N	—	—
18	Female	36.1	24	12.1	Yes	Yes	P	L	N	—	—
20	Female	23.3	13	10.3	No	Yes	G	L	N	—	—
23	Male	51.6	30	21.7	No	Yes	G	R	N	—	—
27	Female	30.7	23	7.9	Yes	No	—	B	Maternal aunt (epilepsy)	—	—
29	Female	53.6	7	46.4	No	Yes	G	L	N	—	—
31	Female	15	1	13.5	No	Yes	G	R	N	Rare burden	*SEC24B*
32	Male	13	9	3.5	No	Yes	—	R	N	—	—
35	Male	55.1	41	14.6	No	Yes	P	L	N	—	—
38	Male	39.3	6	33.3	No	Yes	G	R	N	—	—
42	Female	37.5	7	30.9	No	No	—	R	N	—	—
46	Male	42.3	35	7	Yes	No	—	L	N	—	—
47	Male	28	NA	NA	Yes	Yes	G	L	N	CH	*UBR4*
48	Male	43.6	NA	NA	No	Yes	P	R	N	—	—

Abbreviations: — = not applicable; B = bilateral; CH = compound heterozygous; FS = febrile seizure; L = left; R = right.
[a] G, good outcome with complete seizure freedom after surgery (except on drug withdrawal) and/or complete seizure freedom for 5 years or more at the last follow-up; P, poor outcome with ongoing drug-resistant seizures after surgery. All patients had at least 2 years of follow-up after surgery.
[b] The 3 models of inheritance are de novo, CH and homozygous (homozygous for the same variant), and rare burden (carrying a rare risk variant reported in the gene-based association test).

Table 2 Results of the gene-based association test

Gene	Tested variants	*p* Value	Corrected *p* value
SEC24B	4	8.00×10^{-6}	0.041
SUCO	5	2.70×10^{-5}	0.139
MAN1C1	4	6.15×10^{-5}	0.317
TRDMT1	3	3.83×10^{-4}	1
KIF3A	3	6.12×10^{-4}	1
SS18L1	5	6.19×10^{-4}	1
QSER1	5	6.49×10^{-4}	1
BBS5	3	9.33×10^{-4}	1
CLCN1	7	9.73×10^{-4}	1
ACMSD	3	0.0010	1
GALNTL5	3	0.0010	1
NCKAP5	6	0.0011	1

Genes carrying 3 or more variants were included in the gene-based association tests (5,154 genes tested). The gene-based burden test results of 47 mesial temporal lobe epilepsy related to hippocampal sclerosis cases vs 692 controls.

showed that the NMDA receptor (NMDAR), PSD, FMRP, and metabotropic glutamate receptor (mGluR) 5 gene sets are significantly enriched in the differentially expressed genes (table e-3, links.lww.com/NXG/A55).

Candidate genes in the de novo list were queried against the ClinVar database, and none was known to be associated with epilepsy. The MGI database showed a myriad of genes associated with the abnormal nervous system phenotype in knockout mice. Six of the 18 genes in our de novo list were also listed by MGI, suggesting that their knockout mice model might produce aberrant nervous system phenotypes (*BHLHE40, TACC2, ROBO4, GRASP, BAIAP2,* and *NLGN3*); keywords such as "abnormal nervous system development" and "disrupted synaptic transmission" were frequently observed in the list.

Discussion

In this study, we have identified de novo variants and rare variants possibly involved in the genetic risks of MTLE-HS. Consideration of rare variants in our WES and that of differentially expressed genes in resected hippocampal tissues both suggest that FMRP targets play a potential role in the pathogenesis of MTLE-HS. In addition, we have identified rare variants in *SEC24B*, which might be associated with the disease.[27]

FMRP, encoded by the *FMR1* gene, regulates a number of genes, many of which are expressed in the brain and are implicated in psychiatric disorders.[28] It is important that the *MTOR* gene was reported to be one of the top 5 FMRP-regulated targets; for example, the expression of *FMR1* was shown to reduce the mTOR protein level by 30% in vitro.[20] The mTOR pathway was found to be inactive in sclerotic hippocampus[29] and is believed to induce inflammatory reactions in neurons through the PI3K/Akt/mTOR signaling pathway in patients with MTLE-HS.[30] Our case-control rare variant association study suggested that FMRP targets are significantly associated with MTLE-HS ($p < 3.88 \times 10^{-4}$). It is plausible that the dysregulation of mTOR could be caused by gene mutations of the FMRP pathway. The enrichment test of de novo variants among MTLE-HS cases also suggests that mutations could be introduced to FMRP targets by spontaneous mutations. Both transcriptome and genomic analysis highlighted the importance of FMRP targets in MTLE-HS pathogenesis. However, the size of the FMRP gene set is a potential bias. For the candidate focal epilepsy gene set test, we observed no significant enrichment, but the candidate gene list in table-e1 (links.lww.com/NXG/A55) may be incomprehensive.

Expression data further revealed the complexity of dysregulated pathways related to neural functions in MTLE-HS. In addition to FMRP targets, the results also suggested that mGluR5, PSD, and NMDAR-associated gene sets may play roles in the pathogenesis of MTLE-HS. These gene sets are important for dendritic development and function; hence, they could also be candidate genes of MTLE-HS.

The case-control gene-based association analysis identified *SEC24B* as a potential candidate gene for MTLE-HS. Little is known about the function of this gene, although mutations of *SEC24B* have been reported to cause neural tube development defects in humans and knockout mice.[31] How this relates to the pathogenesis of MTLE-HS is unknown. Targeted studies on our reported candidate genes from the association analysis could be performed to confirm the association signal. Animal models may be used to verify the effect of the observed variants on neural development. Apart from the investigation of well-defined epilepsy subgroups, meta-analysis by aggregating next-generation sequencing studies in consortium settings could be pursued in the light of gaining discovery power. The approach was demonstrated by an International League Against Epilepsy Consortium[5] GWAS studies, which reported associations with genes such as *SCN1A*. However, we did not detect any association signal and de novo variants in the coding region of *SCN1A*. This could be due to the risk that SNPs of MTLE-HS are more frequently found within the promoter region of *SCN1A*. A common variant association study on focal epilepsy also suggested the risk that SNPs of *SCN1A* could act through its expression modulation.[7]

By screening candidate genes carrying de novo variants, we identified another 2 patients carrying compound heterozygous or homozygous rare variants in the gene *CEP170B*. A total of 3 patients carried either inherited or de novo *CEP170B* variants.

Table 3 Results of the gene set burden test

Set[a]	Genes	Damaging *p* value[b]	All *p* value[c]
FMRP-Ascano + FMRP-Darnell	1,557	**2.89×10^{-4}**	**2.15×10^{-3}**
FMRP-Ascano	939	3.15×10^{-3}	**3.88×10^{-4}**
scz-denovo-lof	87	0.013	0.027
ARC complex	28	0.025	0.077
calcium-channel	26	0.033	0.013
FMRP-Darnell	788	0.040	0.450
PSD-95	65	0.040	0.167
PSD	685	0.059	0.303
kirov-denovo-cnv	234	0.118	0.048
mGluR5	39	0.220	0.360
ID-candidates	196	0.289	0.200
ASD-candidates	112	0.357	0.538
FMRP-ASD-overlap-Ascano	93	0.538	1.000
psych-cnv	346	0.538	0.833
ASD-49-gene-network	49	0.583	0.833
scz-denovo-nonsyn	611	0.583	0.120
ASD-74-gene-network	74	0.600	0.282
ID-denovo-nonsyn	132	0.667	0.833
ASD-denovo-nonsyn	743	0.714	1.000
CHD8-network	6	0.714	0.018
miR-137	446	0.714	0.450
scz-gwas	479	0.714	0.370
NMDAR network	61	0.833	0.833
ASD-denovo-lof	128	1.000	0.833
ID-denovo-lof	30	1.000	0.833

Abbreviations: ARC = activity-regulated cytoskeleton-associated protein; ASD = autism spectrum disorder; FMRP = fragile X mental retardation protein; ID = intellectual disability; mGluR = metabotropic glutamate receptor; NMDAR = NMDA receptor; PSD = postsynaptic density.
Bold: significant after multiple testing correction for 48 sets.
The gene set burden test results of 47 mesial temporal lobe epilepsy related to hippocampal sclerosis cases vs 692 controls.
[a] For detail of gene sets, see table e-2, links.lww.com/NXG/A55.
[b] Test on rare and predicted damaging variants.
[c] Test on all rare variants.

Notably, 1 patient who carried compound heterozygous variants on *CEP170B* also showed a family history of MTLE-HS. We could not confirm whether the affected family members also carry 1 or more copies of these *CEP170B* variants, as they have not been tested. The remaining 2 cases affected by recessive *CEP170B* variants appear to be sporadic. De novo variants in *CEP170B* have also been reported in patients with ASD. According to ProteomicsDB,[32] protein expression of *CEP170B* has been detected mostly in the fetal brain and adult brain. The function of *CEP170B* is not well characterized, but the deletion of its paralog *CEP170* is associated with seizures, microcephaly, and corpus callosum abnormalities.[33] Given the number of recurrences and the extensive expression of *CEP170B* in the brain, it is plausible that it may play a role in MTLE-HS pathogenesis.

We found a relatively high ratio of nonsynonymous to synonymous de novo variants, suggesting that de novo variants may be a contributing factor in the pathogenesis of MTLE-HS. Notably, a number of the de novo variants identified overlap with those reported in previous family-based studies of neuropsychiatric disorders. In particular, among the 18 genes carrying the nonsynonymous de novo variants, 3 have been reported in ASD. These include the gain-of-function p.R451C mutation in the esterase domain of NLGN3 (Neuroligin 3).[34] p.R451C mutant mice showed increased AMPA receptor-mediated excitatory synaptic transmission in the hippocampus, raising the amount of NMDA receptors by twofold.[35] In our patient (no. 34), the de novo variant is also found within the esterase domain of NLGN3 (p.S499L). This adds further support to the hypothesis of shared biology between epilepsy and ASD.[27]

One of the patients was found to have a de novo mutation in *GRASP*. Relevant knockout rat models showed reduced dendritic outgrowth in immature hippocampal neurons.[36] The p.N162S mutation carried by our patient maps to the PDZ domain of the GRASP protein, where it binds to mGluRs and gamma-aminobutyric acid B receptor 2. Hence, altered *GRASP* function might affect the development of the hippocampus.

Our study has limitations. The sample size is relatively small, particularly the number of trios. Trios studies are challenging to perform in adults because of logistic reasons (e.g., parents often do not live with probands or unable to participate because of ill health). The possibility of additional extratemporal lobe seizures cannot be completely ruled out in all the patients. However, strict criteria were used to define MTLE-HS, and nearly two-thirds of patients analyzed had histologic confirmation of HS. Parents or controls were not specifically screened for HS by MRI. However, parents or controls with epilepsy were excluded, and the prevalence of HS in people without epilepsy is rare. In a study of 207 patients who underwent high-resolution MRI for nonepilepsy indication (hearing loss), HS was found in 2, both had history of seizures.[37] Hence the prevalence of HS in people without epilepsy is estimated to be less than 0.5%.

This is a study specifically investigating rare variants and de novo variants associated with MTLE-HS, revealing complex genetic architecture. The findings provide further support to the involvement of the PI3K/Akt/mTOR pathway in the pathogenesis of MLTE, potentially via FMRP regulation, and shared pathobiology between epilepsy and other neuropsychiatric disorders. Collaboration effort to increase discovery

Table 4 Genes carrying nonsynonymous de novo variants in probands of trios

Participant no.	Gene	Variant type	Chr	Position	Change	ExAC frequency	Mouse phenotype
9	*FAM65A*	M	16	67574094	g.595C>T:p.R199C	8.3×10^{-6}	NA
12	*FGB*	M	4	155491720	g.1394T>C:p.V465A	0	NA
12	*MASTL*	M	10	27459014	g.1126A>T:p.S376C	0	Abnormal embryonic neuroepithelium morphology
12	***CEP170B***	M	14	105353332	g.2756C>T:p.T919M	1.7×10^{-5}	NA
19	***SLC5A12***	M	11	26743102	g.160G>C:p.G54R	0	Abnormal neuron differentiation, abnormal excitatory postsynaptic potential, reduced long term potentiation
19	*GRASP*	M	12	52407501	g.56A>G:p.N19S	0	Abnormal neuron differentiation, abnormal excitatory postsynaptic potential, reduced long term potentiation
19	*CDC42EP1*	F	22	37962638	g.283delG:p.P95fs	0	NA
24	*NBEAL1*	M	2	203921179	g.335C>G:p.T112S	0	NA
26	*NPC1L1*	M	7	44578845	g.1151C>T:p.S384L	4.9×10^{-5}	NA
30	*ANXA6*	M	5	150481051	g.1980C>G:p.D660E	0	Increased ventricle muscle contractility
34	***NLGN3***	M	X	70387443	g.1496C>T:p.S499L	0	Abnormal CNS synaptic transmission, decreased brain size, abnormal nervous system development
37	*SBSPON*	M	8	74005154	g.149G>T:p.C50F	0	NA
39	*RASEF*	M	9	85615372	g.1551G>T:p.K517N	0	NA
39	***BAIAP2***	M	17	79090095	g.1649C>T:p.A550V	0	Abnormal CNS synaptic transmission
40	***ROBO4***	S	11	124763780	g.1480C>T:p.R494*	5.3×10^{-5}	Abnormal telencephalon development
43	*PLEC1*	M	8	144998224	g.5954C>T:p.T1985M	**0.0004**	Decreased nerve conduction velocity
43	*TACC2*	M	10	123842395	g.380C>T:p.A127V	1.6×10^{-5}	NA
44	*BHLHE40*	M	3	5024745	g.607G>A:p.E203K	0	Increased susceptibility to pharmacologically induced seizures

Abbreviations: ExAC = Exome Aggregation Consortium; F = frameshift; M = missense; NA = not available; S = stop-gain.
Bold: the same gene reported by other de novo variants studies. 1: epileptic encephalopathies, 2: autism spectrum disorder, and 3: schizophrenia.

power and different types of genetic abnormalities (copy number variations [CNVs], methylation) and analysis of the resected hippocampal tissues to identify somatic mutations may further improve our understanding of the pathogenesis of this genetically heterogeneous disorder, potentially leading to novel therapeutic targets.

Author contributions

J.K.L. Wong and H. Gui performed data analysis and prepared the manuscript. M. Kwok performed laboratory work and collected data. P.W. Ng and C.H.T. Lui collected data and provided and cared for study patients. L. Baum advised on data analysis and prepared the manuscript. P.C. Sham served as a scientific advisor. P. Kwan advised on data analysis, prepared the manuscript, and served as a scientific advisor. S.S. Cherny supervised the project, advised on data analysis, prepared the manuscript, and served as a scientific advisor.

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