Novel mutation of SIK1 gene causing a mild form of pediatric epilepsy in a Chinese patient

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Abstract
Developmental and Epileptic Encephalopathy (DEE) is a group of disorders affecting children at early stages of infancy, which is characterized by frequent seizures, epileptiform activity on EEG, and developmental delay or regression. Developmental and epileptic encephalopathy-30 (DEE30) is a severe neurologic disorder characterized by onset of refractory seizures soon after birth or in the first months of life. Which was recently found to be caused by heterozygous mutations in the salt-inducible kinase SIK1. In this study, we investigated a patient with early onset epilepsy. DNA sequencing of the whole coding region revealed a de novo heterozygous nucleotide substitution (c.880G>A) causing a missense mutation (p.A294T). This mutation was classified as variant of unknown significance (VUS) by American College of Medical Genetics and Genomics (ACMG). To further investigate the pathogenicity and pathogenesis of this mutation, we established a human neuroblastoma cell line (SH-SY5Y) stably-expressing wild type SIK1 and A294T mutant, and compared the transcriptome and metabolomics profiles. We presented a pediatric patient suffering from infantile onset epilepsy. Early EEG showed a boundary dysfunction of activity and MRI scan of the brain was normal. The patient responded well to single anti-epileptic drug treatment. Whole-exome sequencing found a missense mutation of SIK1 gene (c.880G>A chr21: 43,420,326 p. A294T). Dysregulated transcriptome and metabolome in cell models expressing WT and MUT SIK1 confirmed the pathogenicity of the mutation. Specifically, we found MEF2C target genes, certain epilepsy causing genes and metabolites are dysregulated by SIK1 mutation. We found MEF2C target genes, certain epilepsy causing genes and metabolites are dysregulated by SIK1 mutation. Our finding further expanded the disease spectrum and provided novel mechanistic insights of DEE30.

Keywords Developmental and epileptic encephalopathy-30 · Early infantile epileptic encephalopathy · Genetic epilepsy · Transcriptome · Metabolome
Introduction

Developmental and epileptic encephalopathy-30 (DEE30) (previously known as SIK1 syndrome) is a newly described developmental epilepsy disorder (OMIM no. 616341) caused by de novo pathogenic sequence variations in the salt-inducible kinase SIK1 (Hansen et al. 2015). SIK1 thus is a novel genetic cause of DEE. Up to date, there are few cases with SIK1 mutations in published literatures and the reported cases present as early myoclonic encephalopathy (EME), Ohtahara syndrome and infantile spasms (Hansen et al. 2015). The DEE30 spectrum and the mechanisms underneath remains poorly explored.

The SIK family includes SIK1-SIK3, which are characterized as serine/threonine kinases belonging to AMP-activated protein kinase (AMPK) family (Chen et al. 2019a, b; Taub et al. 2010). Notably, SIK1 is highly expressed in the adrenal cortex, as well as in the adipose and neural tissues (Chen et al. 2019a, b; Feldman et al. 2000; Horike et al. 2003). ACTH can induce phosphorylation of SIK1, which is dependent on Protein Kinase A (PKA) (Jaleel et al. 2006), resulting in translocation of activated SIK1 from the cytoplasm into the nucleus, where it further phosphorylates HDAC5 (Al-Hakim et al. 2005). Phosphorylation of HDAC5 is required for MEF2C transcriptional activity to proceed (Bertorello and Zhu 2009). MEF2C is known as a transcription factor important in both dorsal and ventral neuronal developmental pathways (Du et al. 2016).

SIKs and their substrates, such as CRTC and class Ila HDAC, are recently reported to be involved in gluconeogenesis (Sakamoto et al. 2018; Wein et al. 2018). SIKs inhibit gluconeogenesis, lipogenesis, steroidogenesis and the production of IL-10 via suppressing various gene expression. Protein and mRNA levels of SIK1 under fasting conditions were increased and SIK1 can inhibit gluconeogenesis in the hepatocytes. Besides, SIK2 inhibits transcriptional repressor ATF3 to upregulate GLUT4 expression, which leads to glucose uptake. Activity of SIKs kinase is regulated by energy deprivation and hormone presence (insulin, glucagon, and ACTH). In addition to its role in glucose metabolism, SIKs also seems to negatively regulate lipid metabolism (Sun et al. 2020). These metabolic roles of SIK1 in the pathogenesis of epilepsy have not been investigated.

Here we presented a pediatric patient suffering from infantile onset epilepsy. Early EEG showed a boundary dysfunction of activity and MRI scan of the brain was normal. The patient responded well to single anti-epileptic drug treatment. Whole-exome sequencing found a missense mutation of SIK1 gene (c.880G>A chr21: 43420326 p. A294T). The pathogenicity of the mutation was investigated by protein stability assay, transcriptome and metabolome profiling in cell models expressing WT and MUT SIK1. Indeed, mutant SIK1 leads to dysregulation of MEF2C target genes, certain epilepsy causing genes and metabolites. These findings strengthened our understanding of DEE30.

Materials and methods

Patient

The ethics committee of Beijing Tiantan Hospital of Capital Medical University approved the study. Written informed consent for participation in the study were obtained from patients’ parents. Blood samples from patient and family members were collected for genetic studies, which were performed in accordance with the Declaration of Helsinki.

Genetic analyses

Genomic DNA were isolated from peripheral blood samples. Disease-causing mutations were screened using whole genome sequencing (WES) (MyGenostics, Inc.). Candidate mutations were confirmed by Sanger sequencing using the following primers: seq F: 5'-CTGACAAGCCGGTGCTGAAGG-3', seq R: 5'-GGTGCGAAGGGGTGCGTGGAAA-3'.

Construction of plasmids

Full length human SIK1 cDNA template (NM173354) was purchased from YouBio (G112872), Shanghai China and subcloned to pLVX-Flag lentiviral expression plasmid and 7.1pcMV-3×Flag expression plasmid as wild type SIK1 (WT) using Seamless Cloning kit from Biomed (CL116), Beijing China. p.A294T mutant of SIK1 was generated by PCR method using WT as template. Mutations were confirmed by Sanger sequencing.

Cells

HEK 293 T (Human Embryonic Kidney 293 cells transformed by expression of the large T antigen from SV40) (ATCC, CRL-11268) were cultured in Dulbecco’s modified Eagle medium (DMEM). SH-SY5Y cells (human ATCC, CRL-2266) were cultured in F12K-DMEM.

Stable cell lines establishment

Lentivirus particles were produced by co-transfected HEK293T cells with pLVX-flag SIK1 WT and mutant
plasmids (2.4 μg), packaging plasmids pCMV-VSV-G (800 ng, AddGene 8454) and psPAX2 (800 ng, AddGene 12260). The medium was changed to fresh DMEM containing 10% FBS at 24 h post transfection and viral supernatant was collected at 48–72 h. Then, a total of 1×10^5 SH-SY5Y cells were infected with viral supernatant supplemented with 8 μg/ml polybrene and incubated for 48 h. Positive cells were screened by puromycin (4 μg/ml) and each monoclone was confirmed by western blot.

**Western blotting**

SH-SY5Y cells were harvested and lysed in RIPA buffer (Beyotime, P0013C), containing complete mini protease inhibitor cocktail (Roche, 04693124001). Proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (GE, PVDF 0.45UM, 10600023). Non-specific binding was blocked using 5% non-fat milk and the primary antibody was incubated at 4°C overnight, the secondary antibody (anti-Flag, Sigma F3165) was incubated at room temperature for 1 h. Signals were visualized by chemiluminescence (Millipore Corporation, Billerica, MA, USA).

**Cycloheximide chase assay**

This assay is adapted from previous report (Kao et al. 2015). Briefly, HEK293T cells were plated and transfected with various WT and mutant 7.1pCMV-3 Flag expression plasmids. 24 h later, cycloheximide was added (1μg/ml) and cells were collected at different time point for western blot detection.

**Transcriptome profiling**

Three monoclones of SH-SY5Y cells stably-expressing WT or Mutant SIK1 were subjected to whole genome RNA-sequencing. RNA was extracted using Trizol (Invitrogen, 15596–026, USA) according to the manufacturer’s protocol. Total RNA was reverse transcribed using a RT-PCR Kit (Tiangen, KR103-03) according to the manufacturer’s protocol. The sequencing reads were generated using the BGISEQ-500 platform following the manufacturer’s recommendations. The paired-end clean reads were aligned to the reference human genome (UCSC version hg19) using TopHat v2.0.12. HTSeq v0.6.1 was used to count the read numbers mapped to each gene and the gene expression levels were calculated with RSEM version v1.2.31. FPKM of each gene was calculated based on the length of the gene and the read count mapped to that gene. We used MA plot, Volcano plot, Scatter plot and Heatmap plot to show the distributions of DEGs. The Holm’s corrected P-value of 0.005 and log2 (fold change) of 1 were set as the threshold for significant differential expression. Then functional enrichment analysis was performed on GeneOntology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

**Immunofluorescence confocal microscopy**

These experiments were performed as described (Hansen et al. 2015) with cell imaging using a Leica TCS SP2 confocal system under a 25× objective.

**Quantitative real-time PCR**

Total RNA was isolated with Trizol reagent (Invitrogen) and then reversed-transcribed with a Reverse Transcription System (Vazyme) following the manufacturer’s protocol. The cDNA was amplified by an ABI 7500 Detection System using the AcqQ qPCR SYBR Green Master Mix (Low ROX Premixed) (Vazyme) and the primers were listed below:

| Gene   | Forward Primer | Reverse Primer |
|--------|----------------|----------------|
| ARC    | AGCGGGACCTGTACACGAC | GCAGGAAACGGTGGTCTCTG | |
| ARC    | GCAAGAAGCCGCGGCTG | CCCCTGAGTTGATCCCTCAC | |
| NR4A1  | GCCCTCAAGGGGTGGAGAAG | GATAAAGGGCTCGCTATTCTGG | |
| NR4A1  | GATCATGGCGTTTTCACGTC | SCN1A  | ATGTGAAATGCTCTGAAAGCAG | |
| NR4A1  | AGCCTACTGAAGGATCCTAC | SCN1A  | AGCCCCAAGGCTAGATCCTACAA | |
| SCN1A  | SCCATCCCACCAATGATTTTG | SCN2A  | TCTAGGCCTGTGTTGCCGCTAAT | |
| SCN2A  | AACCTTCCCATCAAATGATTG | SCN9A  | AGAGGGGTACACCTGTGTTGA | |
| SCN9A  | CCCAGGAAAATCACTACGCAAAA | |

**Metabolome profiling**

Six monoclones of SH-SY5Y cells stably-expressing WT or Mutant SIK1 were subjected to metabolome profiling. LC–MS analysis were performed using a UHPLC system (1290, Agilent Technologies) with a UPLC HSS T3 column (1.8 μm 2.1*100 mm, Waters) coupled to Q Exactive (Orbitrap MS, Thermo). The QE mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during a LC/MS experiment. In this mode, acquisition software (Xcalibur 4.0.27, Thermo)
continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria (Xiao et al. 2012).

MS raw data (.d) files were converted to the mzML format using ProteoWizard, and processed by R package XCMS (version 3.2). The preprocessed results generated a data matrix that consisted of the retention time (RT), mass-to-charge ratio (m/z) values, and peak intensity. Compound Discover (version 2.0, Thermo) and OSI-SMMS (version 1.0, Dalian Chem Data Solution Information Technology Co. Ltd.) was used for peak annotation after XCMS data processing with mz cloud database and in-house MS database (Wang et al. 2014; Xiao et al. 2012).

The peak number, sample name, and normalized peak area were fed to SIMCA14.1 software package (V14.1, MKS Data Analytics Solutions, Umeå, Sweden) for principal component analysis (PCA) and orthogonal projections to latent structures-discriminate analysis (OPLS-DA). PCA showed the distribution of original data. In order to obtain a higher level of group separation and a better understanding of variables responsible for classification, supervised OPLS-DA were applied. sevenfold cross-validation was used to estimate the robustness and the predictive ability of our mode (Wiklund et al. 2008).

Based on OPLS-DA model, a loading plot was constructed, which showed the contribution of variables to the differences between two groups. It also showed the significant variables which were situated far from the origin, but the loading plot is complex because of many variables. To refine this analysis, the first principal component of variable importance in the projection (VIP) was obtained. In addition, commercial databases including KEGG http://www.genome.jp/kegg/ and Metabo Analyst http://www.metaboanalyst.ca were utilized to search for the pathways of metabolites.

Statistical analysis

Samples were compared using two-tailed, unpaired Student’s t-test with GraphPad Prism 7.00. Error bars were represented by SEM. \( *P < 0.05, \quad **P < 0.01, \quad ***P < 0.001 \).

### Results

#### Case description

The patient is a boy over 2 years old with normal spontaneous vaginal delivery and development. He presented the first seizure at 1 year and 3 months without obvious triggering factors. Since then, he experienced 9 more similar seizures in the following 2 months before receiving anti-epileptic treatment. The seizure patterns were all presented as generalized tonic–clonic seizures (GTCS) with 2 of them accompanying with fever of 38 °C.

All the seizures last for about 2–3 min and could remit spontaneously. No obvious abnormality was found on neurological examination and brain magnetic resonance imaging (MRI) (SupFig. 1A). One week later after the last episode, electroencephalogram monitoring showed a moderate increase of background 3-4 Hz δ and θ mixed activity during awake and occasional spike-and-slow waves in bilateral prefrontal regions during sleep. Blood electrolyte, cerebrospinal fluid routine, biochemistry and pathological tests were all normal. The family history of seizure is negative. The patient was diagnosed of epilepsy and treated with sodium valproate. This single anti-epileptic treatment lasted until today (1 year and 3 months) and during follow-up his EEG return to and remain normal. We summarized the antiepileptic drugs used in the reported SIK1 patients in Table 1.

#### Genetic findings

A novel heterozygous \( SIK1 \) mutation (c.880G > A) was detected only in the proband by using whole-exome sequencing, and Sanger sequencing was used to validate it (Fig. 1A). Thus, this is a de novo mutation. This mutation (c.880G > A chr21: 43420326 p. A294T) is identified as “rs776214001” in dbSNP and occurs at a frequency of 0.000042 in the global population, of 0.0000 in Europe, American and African populations and of 0.00020 in Asian population in the ExAC database (https://www.ncbi.nlm.nih.gov/snp/rs776214001?vertical_tab=true#frequency_tab) and has not been

| Patients | Antiepileptic drugs | Literature |
|----------|---------------------|------------|
| P1       | phenobarbital, clonazepam | Hansen et al. 2015 |
| P2       | phenobarbital       | Hansen et al. 2015 |
| P3       | adrenocorticotropic hormone, valproic acid, zonisamide, and clorazepate | Hansen et al. 2015 |
| P4       | phenobarbital, ACTH  | Hansen et al. 2015 |
| P5       | levetiracetam and clobazam | Hansen et al. 2015 |
| P6       | ACTH, phenobarbital, phenytoin, zonisamide, levetiracetam, vigabatrin, rufinamide, valproic acid | Hansen et al. 2015 |
| P7       | sodium valproate    | This paper  |
reported in previous study. Multiple sequence alignment was performed using Mega 7.0, and residue A294 is only highly conserved in primates (Fig. 1B), which may explain that three bioinformatics programs show that the novel mutation might be “Tolerated” (Table 2). The pathogenicity of this variant is classified as variant of unknown significance.
mutations were reported to be responsible for genetic epilepsy with and without seizures (Reynolds et al. 2020). SCN1A and GABRB3 are genes that are associated with autism spectrum disorder and intellectual disability associated with various enrichment methods. It is noteworthy that using DisGeNET enrichment method we found a cluster of dysregulated genes termed “INFANTILE_SEVERE_MYOClonIC_EPILEPSY” (Fig. 2D). Pathogenic variants in SCN2A family, and functions mainly involve in regulating energy response-related physiological processes, such as gluconeogenesis and lipid metabolism (Sakamoto et al. 2018). To investigate the metabolic consequences of SIK1 mutation in our patient, 6 independent monoclonal SH-SY5Y cell lines stably-expressing wild type SIK1 and 6 independent monoclonal SH-SY5Y cell lines stably-expressing p.A294T mutant SIK1. Cells were subjected to nontarget metabolomics profiling and analyzed and compared as WT group and MUT group.

Principal component analysis (PCA) showed a significantly different metabolic pattern between WT group and MUT group (Fig. 3A). Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) (Fig. 3B), which a supervised multivariate statistical analysis with better discriminative power, and Permutation test of OPLS-DA model (Fig. 3C) further confirmed this. Synchronous permutation test can eliminate the overfitting effect of the model.

We then analyzed the differently expressed metabolites between groups and we found there were large number of metabolites upregulated or downregulated in MUT group compared to WT group (Fig. 3D). The top upregulated (Table 3) and downregulated (Table 4) metabolites were listed. Notably, Chondroitin 6-sulfate was significantly increased and Adenosine was significantly decreased in mutant SIK1-expressing cells. The general quantification of differentially expressed metabolites between groups were

SH-SY5Y cells expressing mutant SIK1 showed significantly skewed transcriptome

To gain more insights of the pathogenicity and pathogenesis of patient’s mutation, we infected human neuroblastoma cell line (SH-SY5Y) with a Flag-epitope-tagged wild-type or mutant SIK1 using a lentiviral vector. Next, we established 3 independent monoclonal SH-SY5Y cell lines stably-expressing wild type SIK1 and 3 independent monoclonal SH-SY5Y cell lines stably-expressing p.A294T mutant SIK1. Each cell line achieved robust expression as detected by western blot (Fig. 2A). All 6 cell lines were subjected to bulk RNA-sequencing and analyzed and compared as WT group and MUT group. Pearson correlation between samples showed that within each group samples were closely related (R2 > 92%), while between groups samples were not significantly related (R2 < 80%), indicating transcriptomes within groups are similar but transcriptomes between groups are significantly different (Fig. 2B). The significantly skewed transcriptome of cells expressing p.A294T mutant SIK1 were further supported by the large number of significantly differentially expressed genes (DEGs) as shown in Fig. 2C, with 3178 genes upregulated and 4505 genes downregulated. We next analyzed these DEGs with various enrichment methods. It is noteworthy that using DisGeNET enrichment method we found a cluster of dysregulated genes termed “INFANTILE_SEVERE_MYOClonIC_EPILEPSY” (Fig. 2D). Pathogenic variants in SCN2A can cause spectrum of neurodevelopmental disorders, such as developmental and epileptic encephalopathies, benign familial neonatal-infantile seizures, episodic ataxia, and autism spectrum disorder and intellectual disability with and without seizures (Reynolds et al. 2020). SCN1A mutations were reported to be responsible for genetic epilepsy with febrile seizures plus (GEFS(+)) in multiplex families and accounts for 70–80% of Dravet syndrome (DS) (Marini et al. 2007). The gamma-aminobutyric acid type A receptor β3 gene (GABRB3) encodes the β3-subunit of the gamma-aminobutyric acid type A (GABAA) receptor, and mediate inhibitory signaling within the central nervous system. Recently, GABRB3 mutations have been identified in a few patients with infantile spasms and Lennox–Gastaut syndrome (Papandreou et al. 2016). In addition, other clusters relating to DEE30 disease spectrum were also enriched by this method including “AUTONOMIC_NERVOUS_SYSTEM_DISORDERS” and “AUTISTIC_BEHAVIOR” (Fig. 2E and F). Disease Ontology (DO) enrichment method also showed another cluster of dysregulated genes termed “TEMPORAL_LOBE_EPILEPSY” (Fig. 2G). Further we confirmed some of these dysregulated genes with RT-qPCR which are well known to caused epilepsy (Fig. 2H). Pathogenic mutations of SIK1 were associated with decreased expression of ARC and NR4A1, both are synaptic activity response element genes (Proschel et al. 2017). Consistently, cells expressing p.A294T mutant SIK1 showed decreased expression of ARC and NR4A1 (Fig. 2I), the cellular localization was disrupted in Taken together, these data further confirmed the pathogenicity of the SIK1 mutation of our patient and indicated the mechanism of the epilepsy caused by this mutation might be similar with reported cases at least to certain extent.

SH-SY5Y cells expressing mutant SIK1 showed significantly skewed metabolome

SIKs belong to AMP-activated protein kinase (AMPK) family, and functions mainly involve in regulating energy response-related physiological processes, such as gluconeogenesis and lipid metabolism (Sakamoto et al. 2018). To investigate the metabolic consequences of SIK1 mutation in our patient, 6 independent monoclonal SH-SY5Y cell lines stably-expressing wild type SIK1 and 6 independent monoclonal SH-SY5Y cell lines stably-expressing p.A294T mutant SIK1. Cells were subjected to nontarget metabolomics profiling and analyzed and compared as WT group and MUT group.

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shown in the radar chart (Fig. 3E). Further pathway enrichment analysis showed thiamine and purine metabolism pathways were deeply involved (Fig. 3F).

**Mutant SIK1 do not affect TORC1 or CREB1 protein levels but shows disrupted cellular localization**

TORC1 (CRTC) and CREB1 regulation is a critical role of SIK1 in neurons (Katoh et al. 2006). However, similar to previous report, we did not observe significant difference of protein level between cells expressing WT and mut SIK1 (Fig. 4A). In addition, we found that in contrast to previously reported localization pattern of missense mutant SIK1, which showed a punctate pattern in the nuclei, SIK1 A294T mutant showed a broader pattern of localization within the nucleus and in the cytoplasm, similar to that of nonsense mutants (Fig. 4B) (Hansen et al. 2015).
Discussion

To our best knowledge, only 6 cases of DEE30 have been reported before our report (Hansen et al. 2015), thus our understanding of this disease is rather limited. In this study we presented the seventh case worldwide of DEE30. The clinical and mechanistical investigations we made in our patient added novel knowledge on DEE30.

In the first and only case series report, the authors demonstrated that 3 of 6 cases presented as infantile spasm syndrome, 2 of 6 cases presented as Early Myoclonic Epilepsy (EME) starting within the first hour of life with suppression-burst pattern on EEG and 1 of 6 cases presented as Ohtahara syndrome. These are well known hard-to-treat, very severe forms of epilepsy. Interestingly, our patient, bearing a novel missense mutation of SIK1, showed a mild form of epilepsy and good response to antiepileptic drug treatment. Consistently, EEG test of our patient only showed boundary changes at early disease course and soon returned to normal after antiepileptic drug treatment, while the EEG test of the index patient in the previous report showed burst suppression pattern with myoclonic activity and did not improve after a long period of treatment. The MRI scan of DEE30 patients varied from normal to obvious morphology changes. Our patient showed normal morphology during the whole course.

In addition, 3 previously reported subjects presenting with infantile spasms subsequently developed intractable epilepsy and an autism plus developmental disorder with absent speech, impaired socialization, and repetitive behaviors. Our patient is over 2 years old now and shows no sign of autism nor any other developmental disorders. However, it is noteworthy that the final diagnosis of autism is generally reached until much older. Thus, long term follow-up is needed to fully characterize the clinical phenotype of our patient.

On the aspects of mechanisms, it is reported that all truncated mutant SIK1 showed increased protein stability, abnormal cellular localization and increased activation activity on Histone deacetylase 5 (HDAC5) while all missense mutant SIK1 only showed increased activation activity on HDAC5. In contrast to this, our mutant showed resistance to degradation as previously reported truncated mutants (Fig. 1C). The SIK1 protein is integrated in a complex functional network that relays signaling through protein kinase A to various targets. HDAC5 is one of the best researched targets for SIK1 action (Wein et al. 2018).

Epilepsy-causing SIK1 sequence variations were associated with decreased expression of ARC and other synaptic activity response element genes such as NR4A1 (Proschel et al. 2017). Our result further confirmed this notion (Fig. 21). More interestingly, our transcriptome profiling
| MS2 name                                                                 | MS2 score | MS1 name                                                                 | MS1 ppm   | SuperClass          | Mean mut | Mean wt | P-value      | Q-value      | Fold change |
|--------------------------------------------------------------------------|-----------|---------------------------------------------------------------------------|-----------|---------------------|----------|--------|--------------|--------------|-------------|
| 3-Hydroxy-2-methylpyridine-4,5-dicarboxylate                             | -19.65344592 | 0.08759341                                                             | 0.013345734 | 0.026278367 | 0.045737048 | 6.563401596 |
| Geranoyl-CoA                                                             | -6.26983204 | 0.175250632                                                              | 0.046524568 | 7.4962E-05    | 0.000453269 | 3.766840576 |
| Limocitrin 3-rutinoside                                                  | -20.34597893 | 0.126643516                                                              | 0.03688416 | 1.9180E-06    | 3.5246E-05  | 2.898789383 |
| trans-Dec-2-enoyl-CoA                                                    | -7.19787068 | 0.25501641                                                              | 0.091528874 | 0.000347944 | 0.00135117  | 2.791846764 |
| Octanoyl-CoA                                                             | 17.33389665 | 0.27079088                                                              | 0.105674301 | 0.000218747 | 0.000961042 | 2.56257288  |
| 4-Pyridoxate                                                             | -0.726428636 | 0.013802608                                                             | 0.005648454 | 0.004571927 | 0.001185636 | 2.443680405 |
| Chondroitin 6-sulfate                                                    | 2.248718663 | 0.02087145                                                              | 0.009074711 | 0.032208181 | 0.054015254 | 2.257608528 |
| Octylamine                                                               | 0.985564   | 0.323546321                                                              | 0.143829077 | 0.07223048   | 0.015120091 | 2.249519551 |
| Decanoyl-CoA                                                             | -9.965257107 | 0.260160083                                                              | 0.123261977 | 0.001781746 | 0.004816231 | 2.110627124 |
| 2'-(6-Acetylglucosyl)astragalin                                          | -19.03029101 | 0.19356826                                                              | 0.094479884 | 8.9502E-07  | 2.58441E-05 | 2.048751746 |
| 2,2,6,6-Tetramethyl-4-piperidinone                                       | 0.7600918  | 4.9969969                                                                | 0.158673004 | 0.087740392 | 0.003001404 | 1.808437373 |
| 6-((3-[2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxo-7-([3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy)-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-1-benzyopyran-7-yl]oxy)-3,4,5-trihydroxyoxane-2-carboxylic acid | 4.240818675 | 0.126519997                                                              | 0.048359193 | 0.073985984 | 1.90240555  | 1.224asti-956 |
| Disialyllactose                                                           | -15.14051583 | 0.080879617                                                              | 0.048284228 | 0.017159762 | 0.02147308  | 1.675073202 |
| 6-[(3-[6-(acetylxy)methyl]-3,4,5-trihydroxyoxan-2-yloxy)-5-hydroxy-4-oxo-2-(3,4,5-trihydroxyphenyl)-4H-chromen-7-yl]oxy)-3,4,5-trihydroxyoxane-2-carboxylic acid | 19.69013442 | 0.026152406                                                              | 0.016057098 | 0.002791682 | 0.006849182 | 1.62871323  |
| Cobalamin-precorrin in 4                                                 | -19.36032328 | 0.069139934                                                              | 0.043644284 | 0.010955484 | 0.021754293 | 1.584169278 |
| 3,3'-Dichlorobenzidine                                                   | 13.66528388 | 0.021062997                                                              | 0.013574462 | 0.03469798  | 0.057338069 | 1.531357421 |
| Nicotine                                                                 | 0.9777666  | 0.087092458                                                              | 0.03853246 | 0.041418195 | 0.065830080 | 1.49254175  |
| trans-Zeatin ribose diphosphate                                          | -23.73059867 | 0.169010222                                                              | 0.114509646 | 0.00010257 | 0.000544999 | 1.475963183 |
| 4'-Demethylisholin                                                      | 19.4606933 | 0.129921706                                                              | 0.09713766  | 0.024217792 | 0.042837519 | 1.329415178 |
| Kaempferol 3-(6-acetylexglucosid) 7-rhammoside                           | -10.14565272 | 0.274728893                                                              | 0.218539381 | 0.00395736 | 0.00892018  | 1.257113893 |
| 1-(8-[8]-ladderane-octanoyl-2-(8-[3]-ladderane-octanyl)-sn-glycerol     | 0.3490904   | 0.16340221                                                              | 0.13347385 | 0.011913424 | 0.025734781 | 1.224290656 |
| MS2 name                   | MS2 score | MS1 name                                      | SuperClass                        | Mean mut     | Mean wt     | P-value     | Q-value     | Fold change |
|---------------------------|-----------|-----------------------------------------------|-----------------------------------|--------------|-------------|-------------|-------------|-------------|
| Hypoxanthine              | 0.997795538 | Organoheterocyclic compounds                  |                                   | 0.000196429  | 0.348854784 | 0.000635067 | 0.00212327  | 0.000563068 |
| Ricinoleic Acid methyl ester | 0.934815154 |                                    |                                   | 0.001388963  | 0.096143139 | 0.000177156 | 0.00802721  | 0.014446822 |
| {4-[2,3-dioxo-3-(2,4,6-trihydroxy-3-methoxyphenyl)propyl]-2-methoxy-phenyl}oxidanesulfonic acid | 20.0266141 |                                    |                                   | 0.00927317   | 0.023426969 | 0.004776375 | 0.01058117  | 0.039583308 |
| Epicatechin 3-O-(3-O-methylgallate) | 3.08786925 |                                    |                                   | 0.00903893   | 0.019093862 | 0.00046047  | 0.00166377  | 0.04739451  |
| Stearoylcarnitine         | 0.876258462 | Lipids and lipid-like molecules              |                                   | 0.007127002  | 0.111455391 | 0.000376441 | 0.00143151  | 0.06394488  |
| L-Palmitoylcarnitine      | 0.978753692 | Lipids and lipid-like molecules              |                                   | 0.007581768  | 0.072987999 | 0.000185027 | 0.00085382  | 0.103876907 |
| 7-Ketocholesterol         | 0.985628538 | Lipids and lipid-like molecules              |                                   | 0.054343456  | 0.518420951 | 0.005128861 | 0.01125304  | 0.104824961 |
| 5-Methylthiadenosine      | 14.260215  | Lipids and lipid-like molecules              |                                   | 0.227177776  | 0.231135649 | 0.000263571 | 0.00109183  | 0.119011973 |
| Fe-coproporphyrin III     | 2.99174839 | Lipids and lipid-like molecules              |                                   | 0.046654696  | 0.355497953 | 0.878021E-07| 2.5776E-05  | 0.13237095  |
| Urothion                  | 7.50071205 | Lipids and lipid-like molecules              |                                   | 0.002978519  | 0.022687406 | 3.52199E-05 | 0.00028     | 0.131285143 |
| 3-Oxohexobarbital         | 7.35764298 | Lipids and lipid-like molecules              |                                   | 0.001757901  | 0.013358513 | 9.11501E-06 | 0.00010169  | 0.131549737 |
| ([6-[(2-{3-[3-(6-carboxy-3,4,5-trihydroxyoxan-2-yl)oxy]-4-hydroxyphenyl]-7-hydroxy-5-oxo-5H-chromen-3-yl)oxy]-3,4,5-trihydroxyoxan-2-yl)methyl][3-(3,4-dihydroxyphenyl)-1-hydroxyprop-2-en-1-ylidene]joxidanium | 9.86321823 | Lipids and lipid-like molecules              |                                   | 0.023254124  | 0.174986193 | 0.002423937 | 0.00612641  | 0.132891192 |
| 2,6-Dichloroindophenol    | 15.2185602 | Nucleosides, nucleotides, and analogues      |                                   | 0.049675331  | 0.371757521 | 0.001161439 | 0.0034216   | 0.133622934 |
| Adenosine                 | 0.99929 | Nucleosides, nucleotides, and analogues      |                                   | 0.539074731  | 4.023693682 | 0.000708404 | 0.00230293  | 0.133975092 |
| Telomestatin              | -7.07260018 | Lipids and lipid-like molecules              |                                   | 0.019929945  | 0.137717178 | 6.58716E-09 | 6.467E-06   | 0.144716482 |
| 7-Deoxylate               | 0.76888646 | Lipids and lipid-like molecules              |                                   | 0.025430031  | 0.161114375 | 0.000143897 | 0.00070746  | 0.157818408 |
| Persicachrome             | 0.767663231 | Lipids and lipid-like molecules              |                                   | 0.633712309  | 3.690636323 | 0.00095937  | 0.00119529  | 0.171289156 |
| Sanguirine                | -11.4322868 | Lipids and lipid-like molecules              |                                   | 0.021492378  | 0.125425839 | 8.38034E-05 | 0.00048287  | 0.171355262 |
| MS2 name                        | MS2 score | MS1 name                        | MS1 ppm      | SuperClass | Mean mut | Mean wt | P-value | Q-value | Fold change |
|--------------------------------|-----------|--------------------------------|--------------|------------|----------|---------|---------|---------|-------------|
| Urothion                       | 6.88951114|                                |              |            | 0.032197798 | 0.185040251 | 0.003225202 | 0.00764867 | 0.174004292 |
| Medermycin                     | -21.8738616|                                |              |            | 0.008619372 | 0.048714663 | 0.000271119 | 0.00111643 | 0.176935893 |
| Rabeprazole sodium             | 20.3509592|                                |              |            | 0.002021344 | 0.011153027 | 0.016642796 | 0.03131441 | 0.181237262 |
| Flumequine                     | -13.9484382|                                |              |            | 0.001707947 | 0.009148069 | 0.001401108 | 0.00398456 | 0.186700242 |
| Maculosine                     | 5.0926391 |                                |              |            | 0.100252504 | 0.530816396 | 0.00010443 | 0.00056219 | 0.188864746 |
| Neoacrimarine J                | 4.27836738|                                |              |            | 0.00508701 | 0.026058962 | 1.11775E-06 | 2.6765E-05 | 0.195211538 |
| Luteolin 7-O-[beta-D-glu-        | 16.3413044|                                |              |            | 0.247307272 | 1.241814462 | 0.000396848 | 0.00148706 | 0.199149937 |
| cururonosyl-(1->2)-beta-D-      |           |                                |              |            |          |         |         |         |             |
| glucuronide]-4'-O-beta-D-       |           |                                |              |            |          |         |         |         |             |
| glucuronide                     |           |                                |              |            |          |         |         |         |             |
| Quinoline-3-carboxamides       | 0.66210602|                                |              |            | 0.128386977 | 0.642847874 | 0.000256912 | 0.00107305 | 0.199715955 |
| (2-{5,7-dihydroxy-4-oxo-6-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]}-2-(2,4,5-trihydroxyphenyl)-4H-chromen-8-yl]-3,5-dihydroxy-6-(hydroxymethyl) oxan-4-yl)oxidanesulfonic acid | 12.8868523|                                |              |            | 0.013991002 | 0.069353804 | 0.003166341 | 0.00754326 | 0.201733737 |
| 2-Methylacetoacetyl-CoA         | 19.8836569|                                |              |            | 0.030688084 | 0.152073111 | 4.92124E-05 | 0.00034759 | 0.201798226 |
| Amaroswerin                    | -24.286796|                                |              |            | 0.104123221 | 0.505329582 | 1.82381E-05 | 0.00017487 | 0.20605012 |
| Coenzyme F430                  | 13.1590177|                                |              |            | 0.012503032 | 0.055775476 | 0.002019334 | 0.00531205 | 0.224167198 |
analysis also enriched some well-known epilepsy and autism causing genes in the DEGs dataset, such as SCN1A, SCN2A, SCN9A and GABRB3 (Fig. 2D, E and H), indicating SIK1 plays critical roles in brain function. Indeed, a recent study found SIK1-mutant mice recapitulating the C-terminal-truncated mutations showed increased repetitive behavior and social behavioral deficits, supporting a role of SIK1 in autism pathogenesis (Badawi et al. 2021). In addition, other SIK family members have been shown to be involved in the pathogenesis of depression (Darling and Cohen 2021). It was recently revealed that sodium channel Nav1.5 controls epithelial-to-mesenchymal transition and invasiveness in breast cancer cells through its regulation by the SIK1 (Gradek et al. 2019), indicating the dysregulation of sodium channel by mutant SIK1 might be responsible for the onset of epilepsy in patients with DEE30. The detailed mechanism worth further investigation.

Recently it was found that salt-inducible kinases (SIKs) play critical roles in metabolic homeostasis (Sakamoto et al. 2018). Roles of metabolism in epilepsy are gaining more interest (Patel 2018). Metabolic alterations are commonly seen in various forms of epilepsy syndromes, however, the causal relation between metabolic dysfunction and epilepsy is largely unknown (Patel 2018). In this study, we found epilepsy-causing variant of SIK1 can lead to significant disruption of metabolic homeostasis as demonstrated by the PCA (Fig. 3A), OPLS-DA (Fig. 3B) and vast number of DEGs (Fig. 3D) in the metabolome profiling of cells stably-expressing WT and MUT SIK1. Specifically, cells expressing mutant SIK1 express significantly higher level of Chondroitin 6'-sulfate (Table 3) and lower level of Adenosine compared to cells expressing WT SIK1 (Table 4). Chondroitin sulfate proteoglycans (CSPGs) are predominant components of the extracellular matrix in the central nervous system (CNS). Mice overexpressing chondroitin 6-sulfated chains are more susceptible to seizures (Yutsudo and Kitagawa 2015). Adenosine is a well-characterized endogenous anticonvulsant and seizure terminator of the brain. Through a combination of adenosine receptor-dependent and -independent mechanisms, adenosine affects seizure generation, as well as the development of epilepsy and its progression (Weltha et al. 2019). How does SIK1 contribute to metabolic control of Chondroitin 6'-sulfate and Adenosine also worth further study.

In conclusion, in the present study, we described the seventh case of DEE30 showing a distinct clinical phenotype compared to previously reported cases, which further expanded the spectrum of DEE30. Transcriptome profiling indicated the dysregulation of sodium channel by mutant SIK1 underlie the pathogenesis of DEE30. Metabolome profiling support a potent role of Chondroitin 6'-sulfate and Adenosine in initiating epilepsy. Although more mechanistic details are needed, our study enriched our understanding of DEE30 and epilepsy in general significantly.

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Data availability The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethical Publication Statement We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. The ethics committee of Beijing Tiantan Hospital of Capital Medical University approved the
study. Blood samples collection were performed in accordance with the Declaration of Helsinki.

**Disclosure** None of the authors has any conflict of interest to disclose.

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