Pharmacogenomics and Personalized Medicine

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ORIGINAL RESEARCH

Preliminary Study on Clinical Characteristics and Pathogenesis of *IQSEC2* Mutations Patients

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Background: The IQ motif and Sec7 domain ArfGEF 2 (*IQSEC2*), an X-linked gene that encodes the BRAG1 protein, is a guanine nucleotide exchange factor for the ADP ribosylation factor (ARF) protein family in the small guanosine triphosphate (GTP) binding protein. Mutations in this gene result in disorders such as intellectual disability (ID) and epilepsy. In this study, we analyze the clinical features of two patients with *IQSEC2*-mutation-related disease and discuss their possible pathogenesis.

Methods: The two patients were diagnosed with ID and epilepsy. Genetic testing was performed using whole-exome sequencing, and the three-dimensional protein structure was analyzed. UCSC Genome Browser was used to analyze the conservation of *IQSEC2* in different species. We compared *IQSEC2* expression in the proband families with that in a control group, as well as the expression of the postsynaptic identity protein 95 (PSD-95), synapse-associated protein 97 (SAP97), ADP ribosylation factor 6 (ARF-6), and insulin receptor substrate 53kDa (*IRSP53*) genes interacting with *IQSEC2*.

Results: We identified two semi-zygote mutations located in conserved positions in different species: an unreported *de novo* mutation, C.3576C>A (p. Tyr1192*), and a known mutation, c.2983C>T (p. Arg995Trp). *IQSEC2* mutations resulted in significant changes in the predicted three-dimensional protein structure, while its expression in the two probands was significantly lower than that in the age-matched control group, and *IQSEC2* expression in proband 1 was lower than that in his family members. The expression levels of *PSD-95, ARF-6*, and *SAP97, IRSP 53*, which interact with *IQSEC2*, were also significantly different from those in the family members and age-matched healthy children.

Conclusion: The clinical phenotype resulting from *IQSEC2* mutations can be explained by the significant decrease in its expression, loss of function of the mutant protein, and change in the expression of related genes. Our results provide novel insights into the molecular phenotype conferred by the *IQSEC2* variants.

Keywords: *IQSEC2*, expression level, intellectual disability, infantile spasm, related genes

Introduction

IQSEC2 (NCBI Reference Sequence: NM_001111125.3), located on chromosome Xp11.22, is 6011 bp in size. The transcript contains 15 exons, and BRAG1, the encoded protein, contains 1488 amino acids. *IQSEC2* is named according to its two conserved regions: IQ (347–376) and the SEC7 domain (746–939). The IQ consists of approximately 30 isoleucine and glutamine amino acids and constitutes the initial domain of the IQSEC2 protein and confers calmodulin-binding ability. The SEC7 domain contains approximately 200 amino acids and is responsible for the guanine nucleotide exchange function (GEF). Other functional structures of *IQSEC2* include an N-terminal coiled-coil (CC) domain (23–74), a specific PH domain (951–1085) that binds to inositol phosphate alone, and two C-terminal binding motifs that are crucial in the cell scaffold structure. A proline-rich motif (PRM) (1424–1434) and a PDZ-binding motif (1484–1488) are also present. The PDZ-binding motif is named after the initials of three homologous proteins, namely, postsynaptic density protein 95 (PSD-95), Drosophila disc large tumor suppressor (*DLG1*), and zonula occludens-1 protein (ZO-1).[1]

IQSEC2 interacts with postsynaptic density protein 95 (PSD-95) through its C-terminal PDZ binding domain, forming a complex with N-methyl-D-aspartate (*NMDA*) receptor and allowing for Ca^{2+} influx, which in turn binds to

Received: 25 January 2024
Accepted: 18 May 2024
Published: 29 May 2024

the IQ domain of *IQSEC2*, thereby inducing a conformational change that activates its catalytic domain SEC7. *IQSEC2* acts as a guanine nucleotide exchange factor (GEF) for ADP ribosylation factors (ARFs) through its Sec7 domain, promoting GTP exchange for GDP on ADP ribosylation factor 6 (ARF6) and activating it. The activated *ARF6* then promotes the downregulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors through the stress-activated protein kinase JNK.[1,2] Therefore, *IQSEC2* regulates the membrane structure and synaptic function of neurons. In addition, *IQSEC2* plays an important role in regulating the cell skeleton and vesicle transport density of synapses, making it a key regulator of synaptic plasticity.[3] All necessary for the proper development of cognition and learning. In this study, we compare and analyze the expression of *PSD95, SAP97, ARF6*, and *IRSP 53* in two boys with *IQSEC2* mutations, their family members, and a control group of healthy children of the same age. The mutations were identified as a new nonsense mutation c.3576C>A [p. Tyr1192*] and a missense mutation c.2983C>T [p. Arg995Trp]. Both cases were diagnosed with intellectual disability (ID) and epilepsy, which were consistent with the characteristics of the *IQSEC2* mutations. We also evaluate their clinical features, electroencephalogram (EEG) results, laboratory tests, and molecular characteristics to elucidate the pathogenesis of *IQSEC2* mutations.

Materials and Methods

Variation Detection

From February 2019 to September 2021, the peripheral venous blood of two boys and their nuclear family were collected at Shanghai Children's Hospital. Genomic DNA was isolated from the blood samples of the patients, fragmented using Covaris Ultra Sonicator (Covaris, Inc., MA, USA), used to construct a DNA library, and evaluated for quality. Paired-end sequencing for 150-bp reads was performed on the Hiseq 2500 platform (Illumina, Inc., CA, USA) according to the manufacturer's instructions. Subsequently, the Burrows–Wheeler alignment tool (BWA, version 0.7.15) was used to read the sequencing data and compare it with the NCBI human reference genome (GRCh37/hg19). Common mutations were filtered out based on frequencies (minor allele frequency <0.05) in the Exome Aggregation Consortium (http://exac.broadinstitute.org), the 1000 Genomes Project (http://www.1000genomes.org) database, and the Exome Sequencing Project (https://esp.gs.washington.edu) after the identified variants were filtered and interpreted using Ingenuity Variant Analysis (Qiagen Inc., CO, Germany). The DNA samples of patients and their family members were further sequenced using real-time quantitative polymerase chain reaction (qPCR) and Sanger sequencing.

RNA Extraction and cDNA Synthesis

From each participant 250 μL of blood was collected, and total RNA was extracted using a Qiagen kit (Qiagen Inc., Germany) according to the manufacturer's instructions (Yeasen Biotechnology Co., Ltd., Shanghai, China). The purity and concentration of the total RNA ($A_{260/280}$ ratio is between 2.0) were determined from their OD values measured using a nucleic acid–protein analyzer. Using the total RNA as a template, the reverse transcription cassette was reverse-transcribed using the Script Strand cDNA Synthesis Kit/RT Master Mix (Takara Shuzo Co., Ltd.) according to the manufacturer's instructions. The reaction system was as follows: RNA template, 2 μL (≤500 ng); 5 × gDNA digester mix, 3 μL; RNase free ddH_2O, 10 μL. The sample was centrifuged briefly (8000 rcf, 5 min, 4 °C) and incubated at 42 °C for 2 min. Subsequently, 5 μL of 4×Hifair®III SuperMix plus was added and heated at 25 °C for 5 min, 55 °C for 15 min, and 85 °C for 5 min to inactivate the CorYeabioIIRT Mix.

Analysis of Variance

From the GEO (GENE EXPRESSION OMNIBUS) database (https://www.ncbi.nlm.nih.gov/geo/info/datasets.html) Download and integrate the original mRNA expression data of intellectual disability and epilepsy, and analyze the expression of *IQSEC2* between normal and diseased tissues. The R software package limma (version 3.40.6) was used to analyze the difference in the expression of *IQSEC2*. The ggplot2 package was used to generate a boxplot. The screening threshold was: log FC absolute value ≥ 1, and P<0.05.

Co-Expression Analysis

Download the Series Matrix File data file of GSE31718 from the NCBI GEO public database. The annotation file is GPL6254. Analyze the co expression of *IQSEC2* in the database, and screen 455 genes significantly related to the expression of *IQSEC2*. The correlation coefficient is positive/negative correlation TOP25 genes heat map and co expression correlation circle. The correlation coefficient filter condition is 0.6, and the p value is 0.05. After screening the genes most significantly expressed with *IQSEC2*, the "corrplot" and "circle" packages were used to draw the circle diagram and heat map of *IQSEC2* correlation analysis.

qPCR Analysis of the Expression of IQSEC2 and Its Interacting Genes, PSD-95, SAP97, ARF-6, and IRSP53

We extracted cDNA from the blood samples collected from the two patients and their family members and healthy children of the same age control group (4 boys and 4 girls each, aged between 3–5 years old). Review literature and select four genes significantly correlated with *IQSEC2* expression from heatmaps for experimentation. Then qPCR (Takara Biomedical Technology) was then performed according to the manufacturer's instructions to analyze the relative expression of *IQSEC2* and its interacting genes, *PSD-95, SAP97, ARF-6*, and *IRSP53*, in the family members as well as in the sex- and age-matched control group. The primers used to detect the expression of *IQSEC2* and *PSD-95, SAP97, ARF-6*, and *IRSP53* are listed in Table 1. The reaction mixture (20 μL) contained cDNA (2 μL), forward primer F (10 μM; 1 μL), reverse primer R (10 μM; 1 μL), 2× SYBR Premix Ex Taq (10 μL), and sterilized water (6 μL).

The reaction conditions for qPCR were as follows: pre-denaturation at 95 °C for 30s, 40 cycles of denaturation at 95 °C for 5 s, and renaturation at 60 °C for 20s, followed by a final extension at 65 °C for 15s. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The samples were analyzed in triplicate, repeat the experiment three times per group, from which the average values and their standard errors were calculated. Asterisks indicate statistically significant differences (Student's *t*-test, **P <0.05, ***P <0.01).

Protein Structure Prediction

The I-TASSER server (http://zhanglab.ccmb.med~umich.edu/I-TASSER/) was used to assess missense and nonsense mutations. We predicted the structure of IQSEC2 protein, and its nonsense [c. 3576C>A (P. Tyr1192*)] and missense [c.2983C>T (p. Arg995Trp)] variants. The I-TASSER suite pipeline consisted of four general steps: threading template identification, iterative structure assembly simulation, model selection and refinement, and structure-based functional annotation.

Prediction of RNA Molecular Structure

We used the RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) to predict the secondary structure of RNA molecules with missense mutation c.2983C>T (p. Arg995Trp) and wild-type *IQSEC2* RNA molecules.

Table 1 Specific Primers Used for Quantitative Real-time PCR

Gene	Forward Primer 5′–3′	Reverse Primer 3′–5′
IQSEC2	CCAGAAAGTGGAGCGACTCATC	GCAAGGATGAAGATGGTGTCTGG
PSD-95	TCCACTCTGACAGTGAGACCGA	CGTCACTGTCTCGTAGCTCAGA
SAP97	CGACCTGAAGAATACAGTCGT	GGGATCGCTTCTGGCTAGTTC
ARF-6	ATCTTCGCCAACAAGCAGGA	AGGGCTGCACATACCAGTTC
IRSP53	ATGGAGCAGTTCAACCCTAGC	TTCACCAGGGCGTCAAAGTAG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Conservative Sequence Analysis

We analyzed loci 1192 and 995 in the IQSEC2 protein sequence for humans, *Islupus familiaris, Mus musculus, Rattus norvegicus, Bos taurus, Xenopus tropicalis*, and *Danio rerio* to predict sequence conservation at this locus.

Genetic Pathogenicity Evaluation

The mutation prediction software SIFT (http://sift.jcvi.org), PROVEAN (http://provean.jcvi.org/index.php), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), and Mutation-Taster (http://www.mutationtaster.org) were used to predict the pathogenicity of nonsense [c. 3576C>A (p.Tyr1192*)] and missense [c.2983C>T (p.Arg995Trp)] mutations. The pathogenicity of the two variants was also evaluated using the pathogenicity prediction guide of the American College of Medical Genetics and Genomics (ACMG).

Evaluation of Neurological Function Test Scale

The patients were unable to complete the intelligence test independently. Consequently, the parents aided the evaluation using the neurological function test scale [Warning Signs for Children's Mental and Behavioral Development (WSC-MBD), Autism Behavior Scale (ABC), and social life ability scale (S-M)].

Statistical Analysis

Using the R Programming Language developed by Rick Becker, John Chambers and Allan Wilks exist Bell Labs evaluated the differential expression of *IQSEC2* between the disease group and the control group, and the positive and negative expression of related genes. The $2^{-\Delta\Delta Ct}$ method was used to analyze the expression of *IQSEC2, PSD-95, SAP97, ARF-6* and *IRSP53*. $P<0.05$ is considered to indicate a statistically significant difference.

Results

Clinical Data

Proband 1

Proband 1 was a boy of 5 years and 1 month of age, a second child of unrelated parents delivered at full term by cesarean section, with a birth weight of 4100 g. His older brother was healthy, and his mother experienced a normal pregnancy. At 8 months old, the child presented with difficulties in looking up and sitting alone. Brain nuclear magnetic resonance imaging revealed the possibility of brain dysplasia, and consequently, he started rehabilitation. His parents believed that his condition improved after this treatment. When the child was 2 years and 6 months old, he experienced convulsions, which manifested with strabismus or frequent blinking of both eyes, flexing of both upper limbs, clenching of fists, rigidity and shaking of limbs, and seizures lasting 1–2 min, several times a day. The video electroencephalogram (VEEG) revealed a dominant distribution of extensive or multifocal and slow spikes and six isolated spasms. The patient was diagnosed with Infantile Spasms (IS) and ID. Oral sodium valproate (39 mg/kg/d) was prescribed; however, the treatment outcome was poor.

When the child was 3 years old, he experienced another seizure, characterized by upward eye movement and rapid, brief shaking of both upper limbs, occurring in clusters or isolated incidents, with more than 20 episodes each time.

The blood ammonia and lactic acid levels were 38 μmol/L and 1.5 nmol/L, respectively, which were within the normal range. VEEG results indicated peak rhythm disorder and frequent spasms (Figure 1A). Topiramate (3.125 mg/kg/qn), sodium valproate (40 mL/kg/q12), clonazepam (0.015–0.03 mg/kg/qd), and prednisone acetate (0.6–1 mg/kg/d) were prescribed along with a ketogenic diet (Treatment cycle of 3 months). Reexamination of the VEEG revealed minimal discharge during sleep, and the seizures disappeared (Figure 1B).

Proband 2

Proband 2 was a boy of 4 years and 3 months of age, a fourth child of non-consanguineous parents. His mother gave birth naturally; he weighed 3350 g and has three healthy older sisters. The mother experienced a normal pregnancy. At 6 months of age, his upward gaze was unsteady; at 12 months, he sat alone, albeit unsteadily. His development lagged behind that of children of the same age. At 1 year of age (June 2019), he had convulsions without evident triggers and

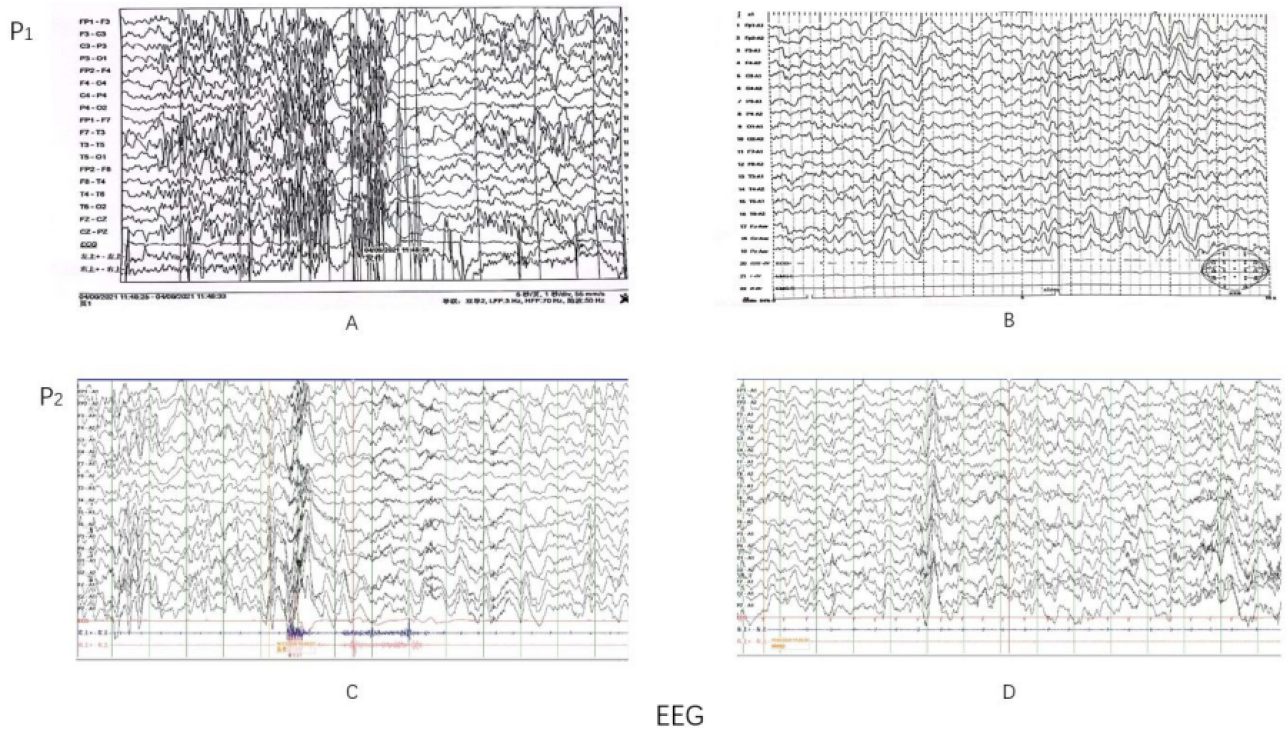

Figure 1 Electrocardiograms of patients (**A**) Proband 1 (P1), April 2021, VEEG: spasms seizure; (**B**) September 2021, VEEG: the seizure disappeared, and peak rhythm disorder disappeared; (**C**) Proband 2 (P2), October 2019, VEEG: spasms seizure; (**D**) August 2021, VEEG: After sleep, the rear head showed epileptiform discharge, and the seizure disappeared.

presented with a fever. His eyes were staring or left oblique, his lips were blue and purple, his limbs were soft, and he could not shout. Each seizure lasted approximately 1 min, and he convulsed 7–8 times a day. VEEG examination at an external hospital indicated peak rhythm disorder and an isolated spasm during sleep. He was diagnosed with Infantile Spasms (IS) and ID, and sodium valproate (43 mg/kg/d) was prescribed. A few weeks later, the number of seizures increased, and the pattern eventually changed. The seizures manifested as a series of nodding attacks, sometimes accompanied by body impact. The patient was admitted to our hospital in October 2019. The blood ammonia levels and the tandem mass spectrometry results were normal. VEEG results indicated a peak rhythm disorder, with seven spasms during waking and sleeping (Figure 1C). Topiramate (4 mg/kg/d) was prescribed along with adrenocorticotropic hormone ACTH (25 U/d) for three weeks, and subsequently, prednisone acetate (0.8–1.2 mg/kg/d) was administered orally. A reexamination of the VEEG in 2021 revealed intermittent epileptiform discharges during sleep; however, no seizures were detected (Figure 1D).

Variant Detection

Proband 1

The whole exon sequencing revealed a human genome version: Human GRCh37/hg19 (NM_001111125.3) c.3576C>A (p.Tyr1192*) hemizygote mutation in *IQSEC2*, which is harbored in exon 15 of the X chromosome in proband 1. This variation introduced a pretermination stop codon at amino acid 1192, resulting in the early termination of protein translation. However, Sanger sequencing results confirmed that the parents and brother of proband 1 did not carry the mutation, and thus, it was classified as *de novo* (Figure 2A).

Proband 2

The whole exon sequencing helped identify the human genome version: Human GRCh37/hg19 (NM_001111125.3) c.2983C>T (p. Arg995Trp) hemizygote variation in exon 10 of *IQSEC2* in proband 2. This mutation resulted in an amino

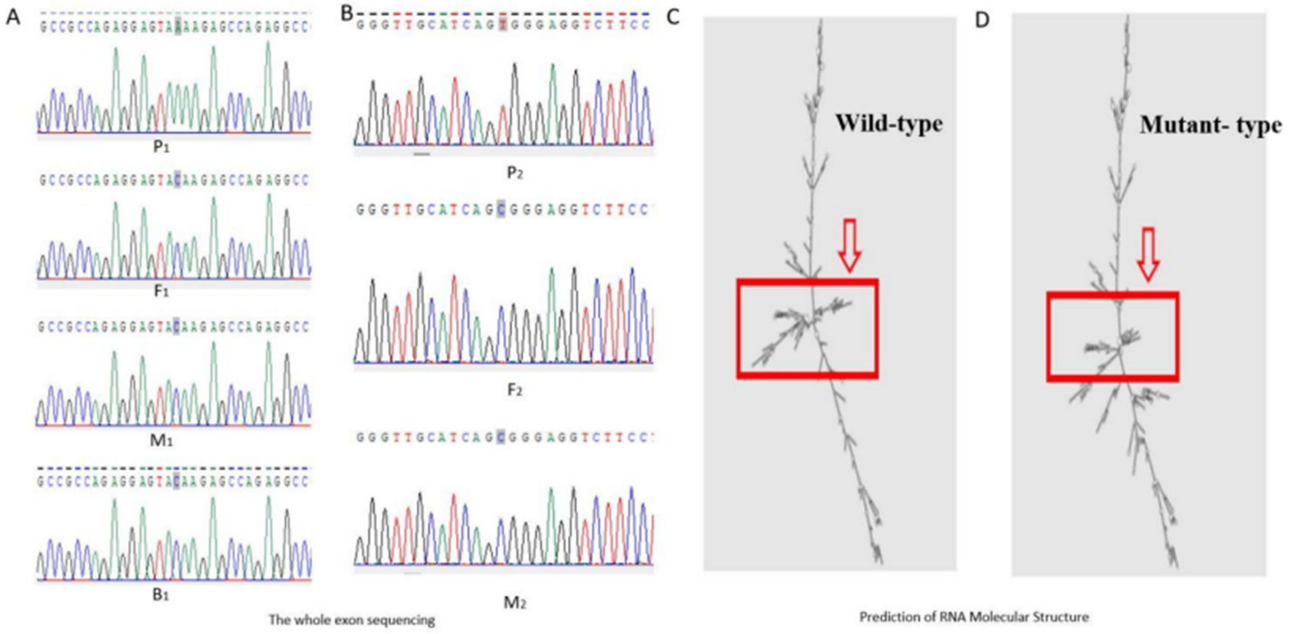

Figure 2 (**A**) The sequences of genomic DNA suggest that the nonsense mutation of *IQSEC2* c.3576C>A (p. Tyr1192*) was identified in proband 1 but not in his parents and brothers. (**B**) Missense mutation of *IQSEC2* c.2983C>T (p. Arg995Trp) in proband 2 but not in his parents. (**C**) Secondary molecular structures of *IQSEC2* RNA Wild-type. (**D**) missense mutant RNA.

acid substitution from arginine to tryptophan at position 995. The Sanger sequencing results confirmed that the parents of proband 2 did not carry mutations, and hence were classified as *de novo* (Figure 2B).

Results of the Analysis of Variance and qPCR Analysis of the Transcription Level of IQSEC2

Download the Series Matrix File data file of GSE185031 from the NCBI GEO public database, and included the expression profile data of 14 patients, including 5 cases in the normal group and 9 cases in the disease group. Used for analyzing the differences between *IQSEC2* and the normal group. The results showed that the expression of *IQSEC2* was lower than that of the normal control group (Figure 3A). The expression levels of *IQSEC2* in probands 1 and 2 were significantly lower than those in their core families and control group (P <0.01) (Figure 3B).

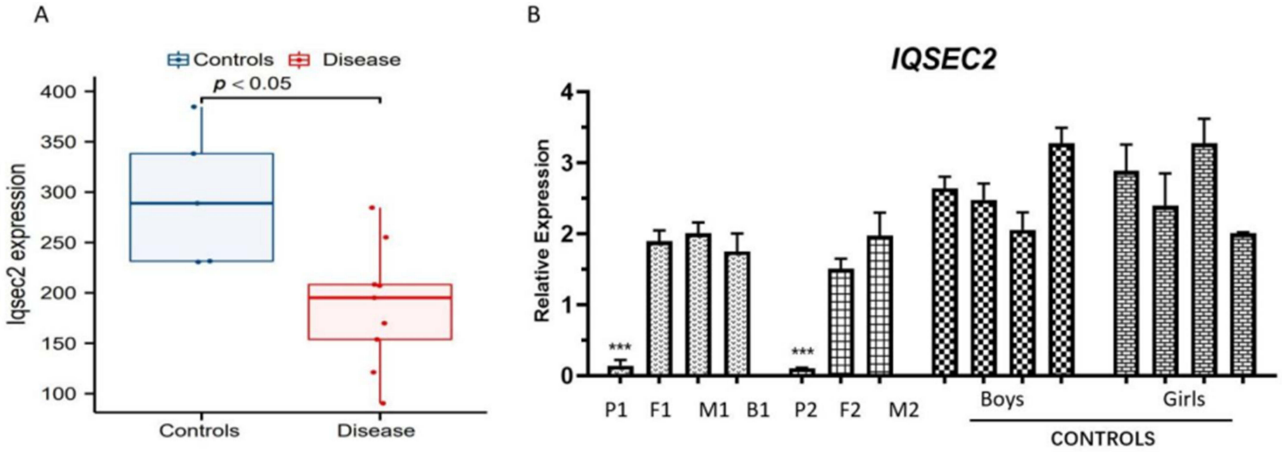

Figure 3 (**A**) Analysis of variance between *IQSEC2* and the normal group. The left vertical axis represents gene expression levels, the red box plot represents gene expression levels in the disease group, and the blue box plot represents gene expression levels in the control group. The results showed that the expression of *IQSEC2* was lower than that of the normal control group. (**B**) qPCR of *IQSEC2*. In proband 1 (P1) and proband 2 (P2), the expression was significantly lower than that of their core family members and normal control group.

Co-Expression Analysis

We further explored the co expression network of *IQSEC2* through correlation analysis based on the expression profile of epileptic patients in GEO database, with 385 up-regulated and 70 down-regulated. The filter condition of correlation coefficient is 0.6, and the p value is 0.05. A total of 455 genes significantly related to the expression of *IQSEC2* were screened (Table 2), and the heatmap of genes with positive/negative correlation coefficients with *IQSEC2* (Figure 4A) and the circular graph of co expression with *IQSEC2* (Figure 4B).

Table 2 Genes co expressed with *IQSEC2*

Query	Gene	cor	pvalue
IQSEC2	*ABCC5*	0.687554454	0.000808204
IQSEC2	*ABTB1*	0.6144283	0.003945944
IQSEC2	*ADAMTS2*	-0.603890044	0.004807887
IQSEC2	*ADCK5*	0.65310929	0.001794879
IQSEC2	*ADCY2*	0.633927858	0.002687229
IQSEC2	*ADCYAP1R1*	0.622605743	0.003368985
IQSEC2	*ADRA1D*	0.635031286	0.00262744
IQSEC2	*AGPAT2*	0.601183041	0.005052738
IQSEC2	*AHDC1*	0.620157205	0.003533867
IQSEC2	*AKAP3*	0.719575256	0.000348253
IQSEC2	*AKR7A2*	-0.614105161	0.003970324
IQSEC2	*ALDH9A1*	-0.722911702	0.000316958
IQSEC2	*ALKBH6*	0.717579003	0.000368206
IQSEC2	*ALKBH7*	0.68893181	0.000781126
IQSEC2	*ALKBH8*	0.634916268	0.00263362
IQSEC2	*ANKRD34*	0.673046723	0.00114505
IQSEC2	*ANKRD39*	0.629435324	0.002942494
IQSEC2	*ANTXR1*	-0.659610035	0.001555665
IQSEC2	*ANXA6*	0.63777936	0.002483336
IQSEC2	*AOF1*	-0.699029738	0.000605042
IQSEC2	*AP1S3*	0.710004036	0.000453029
IQSEC2	*AP2B1*	0.666932075	0.001318852
IQSEC2	*APBB1*	0.604610546	0.004744394
IQSEC2	*APPBP2*	-0.641159342	0.002315195
IQSEC2	*AQP5*	0.649444834	0.001942798
IQSEC2	*ARD1B*	0.761792746	9.51E-05
IQSEC2	*ARF6*	0.720673311	0.000337675
IQSEC2	*ARFRP1*	0.691746084	0.000728178

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*ARHGAP26*	0.630329408	0.002890146
IQSEC2	*ARHGEF17*	0.680925413	0.0009499
IQSEC2	*ARL4C*	0.607886383	0.004464377
IQSEC2	*ARL4D*	0.641360865	0.002305478
IQSEC2	*ARMC9*	0.618641644	0.003639237
IQSEC2	*ARX*	0.622538709	0.003373412
IQSEC2	*ASPHD1*	0.648525997	0.001981446
IQSEC2	*ASPHD2*	0.651054388	0.001876618
IQSEC2	*ASRGL1*	0.600324376	0.005132518
IQSEC2	*ATG4D*	0.646727531	0.002058956
IQSEC2	*ATM*	0.601004435	0.005069248
IQSEC2	*ATP13A2*	0.7227072	0.000318805
IQSEC2	*ATP1A3*	0.675003122	0.001093693
IQSEC2	*ATP6V0A1*	0.656501524	0.001666476
IQSEC2	*B4GALNT1*	0.684967901	0.000861202
IQSEC2	*B4GALT7*	0.808800547	1.58E-05
IQSEC2	*BAI2*	0.725151799	0.000297321
IQSEC2	*BAIAP2*	0.748561033	0.000146664
IQSEC2	*BAX*	0.614551772	0.003936661
IQSEC2	*BCAS4*	0.646762517	0.002057424
IQSEC2	*BCR*	0.625932872	0.00315522
IQSEC2	*BOLA3*	-0.685750401	0.000844868
IQSEC2	*BRUNOL4*	0.724534214	0.000302628
IQSEC2	*BSCL2*	0.657568948	0.001627702
IQSEC2	*BSG*	0.682510274	0.000914249
IQSEC2	*BTBD2*	0.736424766	0.000213476
IQSEC2	*C10orf114*	0.632381973	0.002772897
IQSEC2	*C11orf80*	0.606472322	0.004583526
IQSEC2	*C12orf52*	0.674896379	0.001096444
IQSEC2	*C12orf53*	0.702851902	0.000547824
IQSEC2	*C13orf8*	-0.665227523	0.001371078
IQSEC2	*C14orf79*	0.627434759	0.003062487
IQSEC2	*C16orf3*	0.65818288	0.001605746

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*C16orf77*	0.61923527	0.003597658
IQSEC2	*C17orf46*	0.62023348	0.003528631
IQSEC2	*C17orf70*	0.641515443	0.002298048
IQSEC2	*C19orf22*	0.684807162	0.00086459
IQSEC2	*C1QTNF4*	0.730946357	0.000251269
IQSEC2	*C1orf115*	0.620648755	0.00350024
IQSEC2	*C1orf131*	-0.63665413	0.002541523
IQSEC2	*C1orf70*	0.633372008	0.002717775
IQSEC2	*C1orf89*	0.652556638	0.001816563
IQSEC2	*C20orf199*	-0.658137641	0.001607355
IQSEC2	*C21orf100*	-0.649043844	0.001959586
IQSEC2	*C21orf56*	0.667162008	0.001311937
IQSEC2	*C22orf36*	0.635474178	0.002603755
IQSEC2	*C2orf15*	0.623320528	0.003322075
IQSEC2	*C2orf44*	-0.608714015	0.004395836
IQSEC2	*C7orf27*	0.685881183	0.000842163
IQSEC2	*C8orf16*	0.668548258	0.00127089
IQSEC2	*C8orf80*	0.660712261	0.001517889
IQSEC2	*C9orf91*	-0.603816372	0.004814419
IQSEC2	*CAMK1*	0.60631848	0.004596646
IQSEC2	*CAMK1G*	0.600404813	0.005125001
IQSEC2	*CAMKK2*	0.689186274	0.000776209
IQSEC2	*CAPN1*	0.702995151	0.000545772
IQSEC2	*CAPN10*	0.622726752	0.003361005
IQSEC2	*CAPNS1*	0.605109356	0.004700844
IQSEC2	*CCDC101*	0.762090226	9.41E-05
IQSEC2	*CCDC24*	0.650393553	0.001903557
IQSEC2	*CCDC26*	0.622271207	0.003391128
IQSEC2	*CCDC48*	-0.61802911	0.003682556
IQSEC2	*CCDC92*	0.646288405	0.002078262
IQSEC2	*CCL26*	-0.630461264	0.002882492
IQSEC2	*CCNT1*	0.641508487	0.002298382

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*CDC42BPB*	0.691194737	0.000738305
IQSEC2	*CDH4*	0.78711092	3.82E-05
IQSEC2	*CDH7*	0.620230321	0.003528848
IQSEC2	*CDIPT*	0.718465139	0.000359232
IQSEC2	*CDK10*	0.679540696	0.000982003
IQSEC2	*CDK5*	0.686074527	0.000838179
IQSEC2	*CECR6*	0.715039424	0.000394991
IQSEC2	*CEND1*	0.679381156	0.00098576
IQSEC2	*CENTB5*	0.639689344	0.002387114
IQSEC2	*CHRM1*	0.754475861	0.000121222
IQSEC2	*CHRM3*	0.632800948	0.002749457
IQSEC2	*CHRNA6*	0.616358388	0.003802891
IQSEC2	*CKB*	0.616063061	0.003824497
IQSEC2	*CLASP1*	0.673727227	0.00112696
IQSEC2	*CLN8*	0.640876656	0.002328883
IQSEC2	*CLOCK*	0.675442045	0.001082442
IQSEC2	*CLSTN3*	0.658245754	0.001603511
IQSEC2	*CNIH2*	0.718897738	0.000354919
IQSEC2	*COASY*	0.656604364	0.001662707
IQSEC2	*COL23A1*	0.709294435	0.000461763
IQSEC2	*COL4A3BP*	0.648208337	0.001994956
IQSEC2	*CORO1A*	0.601461455	0.00502709
IQSEC2	*CPSF3L*	0.676947945	0.001044578
IQSEC2	*CRHR1*	0.697092709	0.000635923
IQSEC2	*CRYBB2*	0.72436489	0.000304097
IQSEC2	*CSPG5*	0.642440038	0.00225402
IQSEC2	*CUL4B*	-0.704338686	0.000526843
IQSEC2	*CX3CL1*	0.692854806	0.00070817
IQSEC2	*CXXC4*	0.703713636	0.000535579
IQSEC2	*CYB561D1*	0.647311833	0.0020335
IQSEC2	*CYC1*	0.603561731	0.004837051
IQSEC2	*CYHR1*	0.67405364	0.00111837

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*DAPK3*	0.609024918	0.004370314
IQSEC2	*DDX46*	-0.634588327	0.002651307
IQSEC2	*DEPDC5*	0.648249514	0.0019932
IQSEC2	*DERA*	-0.650108183	0.00191529
IQSEC2	*DGAT1*	0.682607974	0.000912089
IQSEC2	*DGCR14*	0.660809701	0.001514587
IQSEC2	*DHRS12*	-0.671300362	0.001192594
IQSEC2	*DIRAS1*	0.679992969	0.000971419
IQSEC2	*DLG4*	0.679861921	0.000974476
IQSEC2	*DLX5*	0.65940135	0.001562905
IQSEC2	*DNAJC14*	0.629076943	0.002963697
IQSEC2	*DOC2A*	0.701365152	0.000569509
IQSEC2	*DOLK*	0.66137814	0.001495444
IQSEC2	*DPF1*	0.723375994	0.0003128
IQSEC2	*DPM2*	0.666419449	0.001334379
IQSEC2	*DPM3*	0.642286775	0.002261269
IQSEC2	*DRD1IP*	0.657514365	0.001629666
IQSEC2	*DUSP13*	0.60113518	0.005057158
IQSEC2	*DVL1*	0.613003072	0.004054414
IQSEC2	*DZIP3*	0.603042841	0.004883441
IQSEC2	*ECHDC2*	0.61487492	0.003912452
IQSEC2	*EDN2*	-0.725141042	0.000297412
IQSEC2	*EGFL7*	0.721995937	0.000325299
IQSEC2	*EHBP1L1*	0.690664979	0.000748147
IQSEC2	*ETV6*	0.623159949	0.003332566
IQSEC2	*EVI5L*	0.735539563	0.000219231
IQSEC2	*EXOC3*	-0.641914195	0.002278973
IQSEC2	*EXTL1*	0.693680536	0.000693573
IQSEC2	*EXTL3*	0.709040585	0.000464922
IQSEC2	*FABP3*	0.685209676	0.000856127
IQSEC2	*FAM101A*	0.64659419	0.002064802
IQSEC2	*FAM116B*	0.721725403	0.000327799
IQSEC2	*FAM5B*	0.613770751	0.003995686

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*FAM65A*	0.656050626	0.001683086
IQSEC2	*FAM98C*	0.762133122	9.40E-05
IQSEC2	*FARSA*	0.745830462	0.000159874
IQSEC2	*FBXL15*	0.707781067	0.000480868
IQSEC2	*FLOT2*	0.632952991	0.002740992
IQSEC2	*FNTA*	-0.623043461	0.003340193
IQSEC2	*FOXJ2*	0.619196559	0.003600358
IQSEC2	*FRAT2*	-0.623059173	0.003339164
IQSEC2	*FREQ*	0.63658452	0.002545159
IQSEC2	*FSD1*	0.714836749	0.000397198
IQSEC2	*FXYD6*	0.643211593	0.002217822
IQSEC2	*GABRA2*	0.634084587	0.002678668
IQSEC2	*GABRB3*	0.643530892	0.002202984
IQSEC2	*GCSH*	-0.640826817	0.002331303
IQSEC2	*GDF15*	-0.609163094	0.004359011
IQSEC2	*GGA2*	0.626309757	0.003131733
IQSEC2	*GGT6*	-0.647040608	0.002045284
IQSEC2	*GIMAP1*	0.604016256	0.004796715
IQSEC2	*GIMAP4*	-0.64361845	0.00219893
IQSEC2	*GIT1*	0.756134859	0.000114806
IQSEC2	*GLB1L*	0.61656461	0.003787863
IQSEC2	*GLDC*	0.660146847	0.00153717
IQSEC2	*GNA11*	0.673544167	0.001131803
IQSEC2	*GNAO1*	0.609787212	0.004308256
IQSEC2	*GNG3*	0.664904133	0.001381179
IQSEC2	*GPR128*	-0.681332836	0.000940625
IQSEC2	*GPR175*	0.683739876	0.000887374
IQSEC2	*GPS1*	0.602153644	0.004963791
IQSEC2	*GRIA3*	0.636204988	0.002565063
IQSEC2	*GRN*	0.645880962	0.002096309
IQSEC2	*H1FX*	0.675658228	0.001076936
IQSEC2	*HAS2*	-0.605428144	0.004673185
IQSEC2	*HBD*	-0.644224279	0.002171049

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*HD*	0.672376292	0.00116311
IQSEC2	*HDHD3*	0.645669159	0.002105743
IQSEC2	*HERPUD2*	-0.605238492	0.004689624
IQSEC2	*HES4*	0.672450932	0.001161087
IQSEC2	*HOXA9*	-0.632061878	0.002790916
IQSEC2	*HRAS*	0.703746095	0.000535122
IQSEC2	*HRH3*	0.697344959	0.000631827
IQSEC2	*HSD17B14*	0.615043757	0.003899852
IQSEC2	*HSPE1*	-0.616945608	0.00376023
IQSEC2	*HTR2A*	0.628492135	0.002998569
IQSEC2	*HYOU1*	0.630602007	0.002874341
IQSEC2	*IARS2*	-0.703631329	0.000536738
IQSEC2	*IFT122*	0.657760868	0.001620811
IQSEC2	*IGSF21*	0.672999344	0.001146318
IQSEC2	*IL12RB2*	0.651809124	0.001846242
IQSEC2	*ING4*	0.61346669	0.004018862
IQSEC2	*INHBE*	-0.607921948	0.004461414
IQSEC2	*INTS1*	0.650821419	0.001886078
IQSEC2	*INTS5*	0.697654099	0.000626838
IQSEC2	*ITFG3*	0.683532787	0.000891853
IQSEC2	*ITGBL1*	-0.604697183	0.004736806
IQSEC2	*KATNAL2*	-0.620050807	0.00354118
IQSEC2	*KCNC1*	0.64008736	0.00236746
IQSEC2	*KCNN1*	0.716589076	0.000378457
IQSEC2	*KCTD17*	0.654845345	0.001728168
IQSEC2	*KIAA1274*	0.626707468	0.003107106
IQSEC2	*KIAA1328*	-0.657963254	0.001613571
IQSEC2	*KIAA1543*	0.700466629	0.000582965
IQSEC2	*KIAA1751*	0.630989316	0.002852008
IQSEC2	*KIFC3*	0.643786416	0.00219117
IQSEC2	*KLC2*	0.619361034	0.003588901
IQSEC2	*KLHDC8A*	0.685755114	0.00084477
IQSEC2	*KLHDC8B*	0.727962649	0.000274155

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*KLHL22*	0.720958279	0.000334975
IQSEC2	*KRT73*	-0.620273261	0.003525903
IQSEC2	*LEPREL2*	0.737116591	0.000209069
IQSEC2	*LINGO1*	0.759822813	0.00010158
IQSEC2	*LIPG*	-0.667962258	0.001288108
IQSEC2	*LMAN2*	0.703227359	0.00054246
IQSEC2	*LMNB1*	0.641836692	0.00228267
IQSEC2	*LRRC4B*	0.644398067	0.002163106
IQSEC2	*LRRIQ1*	0.616262277	0.003809911
IQSEC2	*LTBP3*	0.696042223	0.000653221
IQSEC2	*LY6E*	0.71026667	0.000449832
IQSEC2	*LY6H*	0.693121605	0.000703425
IQSEC2	*LYPLA3*	0.647128285	0.002041468
IQSEC2	*MADD*	0.690968948	0.000742486
IQSEC2	*MAEL*	0.63462622	0.002649258
IQSEC2	*MAF1*	0.636115975	0.00256975
IQSEC2	*MAGEB10*	-0.605957521	0.004627551
IQSEC2	*MAN2C1*	0.621704975	0.003428882
IQSEC2	*MAP2K5*	0.605255152	0.004688178
IQSEC2	*MAP2K6*	0.659055402	0.001574969
IQSEC2	*MAPKBP1*	0.730077182	0.000257761
IQSEC2	*MAPT*	0.708595552	0.000470504
IQSEC2	*MARCKS*	0.661087589	0.001505203
IQSEC2	*MAST4*	0.644467897	0.002159921
IQSEC2	*MBD3*	0.65461724	0.001736813
IQSEC2	*MC5R*	-0.693634946	0.000694372
IQSEC2	*MCAT*	0.638515472	0.002445876
IQSEC2	*MEGF8*	0.656518854	0.00166584
IQSEC2	*MEN1*	0.625396902	0.003188873
IQSEC2	*MESP1*	0.621092857	0.003470088
IQSEC2	*MFSD2*	0.618734545	0.003632704
IQSEC2	*MFSD5*	0.624387949	0.003253033
IQSEC2	*MICALCL*	0.71669785	0.000377319

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*MIDN*	0.616001038	0.003829047
IQSEC2	*MIER1*	-0.626548933	0.003116903
IQSEC2	*MLXIPL*	0.639200824	0.002411424
IQSEC2	*MNDA*	-0.655558657	0.001701367
IQSEC2	*MPG*	0.675660426	0.00107688
IQSEC2	*MRPL12*	0.656177662	0.001678392
IQSEC2	*MRPL23*	0.741898638	0.000180679
IQSEC2	*MRPL28*	0.709480613	0.000459457
IQSEC2	*MRPS12*	0.702844662	0.000547928
IQSEC2	*MTHFS*	-0.604936853	0.004715868
IQSEC2	*MYL6B*	0.652803847	0.001806836
IQSEC2	*MYLK*	0.630076619	0.002904867
IQSEC2	*NAT14*	0.741016884	0.00018565
IQSEC2	*NCAN*	0.62066165	0.003499362
IQSEC2	*NCLN*	0.683284351	0.000897251
IQSEC2	*NDUFS4*	-0.609499458	0.004331596
IQSEC2	*NDUFV1*	0.629663023	0.002929088
IQSEC2	*NFRKB*	0.646713262	0.002059581
IQSEC2	*NGB*	0.699679524	0.000594972
IQSEC2	*NKD1*	-0.697311159	0.000632375
IQSEC2	*NPPA*	0.687361562	0.000812059
IQSEC2	*NRSN2*	0.616509016	0.00379191
IQSEC2	*NTSR1*	0.693002727	0.000705536
IQSEC2	*NUDT13*	-0.66570446	0.001356293
IQSEC2	*NUDT18*	0.710380936	0.000448447
IQSEC2	*NUMBL*	0.717879744	0.000365139
IQSEC2	*NUP62*	0.616421886	0.003798258
IQSEC2	*NUTF2*	0.610763523	0.00422984
IQSEC2	*NXN*	0.748810843	0.000145504
IQSEC2	*PACS1*	0.772881919	6.47E-05
IQSEC2	*PALM*	0.680219205	0.00096616
IQSEC2	*PAPD5*	0.67934259	0.00098667
IQSEC2	*PCLO*	0.78432548	4.25E-05

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*PCMTD1*	-0.627274058	0.0030723
IQSEC2	*PDE2A*	0.676205572	0.001063102
IQSEC2	*PDE4C*	0.672476094	0.001160406
IQSEC2	*PDE8B*	0.621411183	0.003448608
IQSEC2	*PFKL*	0.672304442	0.001165059
IQSEC2	*PGLS*	0.620955972	0.003479359
IQSEC2	*PHPT1*	0.657825986	0.001618479
IQSEC2	*PHYH*	-0.707756714	0.000481181
IQSEC2	*PHYHIP*	0.656561234	0.001664286
IQSEC2	*PIP5K1C*	0.628627684	0.002990456
IQSEC2	*PLCG1*	-0.614677674	0.003927214
IQSEC2	*PLD3*	0.718632225	0.000357561
IQSEC2	*PLEKHJ1*	0.649448824	0.001942631
IQSEC2	*PLTP*	0.653859633	0.001765786
IQSEC2	*PNPLA2*	-0.643399279	0.00220909
IQSEC2	*POLR1E*	-0.610030704	0.004288587
IQSEC2	*POLR3H*	0.633754384	0.002696731
IQSEC2	*PON2*	-0.726750671	0.000283947
IQSEC2	*PPARD*	0.658168856	0.001606244
IQSEC2	*PPCDC*	-0.627129913	0.003081124
IQSEC2	*PPP2R3C*	-0.609887948	0.00430011
IQSEC2	*PPP2R4*	0.641938428	0.002277818
IQSEC2	*PRDM15*	0.654711513	0.001733235
IQSEC2	*PRDM2*	0.646397851	0.002073436
IQSEC2	*PRELID1*	0.709815785	0.000455332
IQSEC2	*PRKAR1B*	0.643408979	0.00220864
IQSEC2	*PRKCSH*	0.692225647	0.000719466
IQSEC2	*PRR4*	-0.620207318	0.003530426
IQSEC2	*PRR7*	0.704371232	0.000526392
IQSEC2	*PSD95*	0.702358389	0.000554943
IQSEC2	*PSG9*	-0.786267894	3.95E-05
IQSEC2	*PTGES2*	0.705617554	0.000509346
IQSEC2	*PTMS*	0.714391007	0.00040209

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*PTPN5*	0.674394492	0.001109458
IQSEC2	*PVRL3*	0.601941665	0.004983106
IQSEC2	*PWWP2*	0.615613431	0.003857587
IQSEC2	*RAB26*	0.656023097	0.001684104
IQSEC2	*RAB9B*	0.650374338	0.001904345
IQSEC2	*RABAC1*	0.610219627	0.004273378
IQSEC2	*RBM9*	0.709296544	0.000461736
IQSEC2	*RDH13*	0.626055615	0.003147555
IQSEC2	*RHBDD2*	0.668628505	0.001268547
IQSEC2	*RHBDL1*	0.775422351	5.91E-05
IQSEC2	*RHOBTB2*	0.674239536	0.001113502
IQSEC2	*RHOT2*	0.690041146	0.000759879
IQSEC2	*RHPN1*	0.658064583	0.001609957
IQSEC2	*RIF1*	0.628815372	0.002979252
IQSEC2	*RND1*	0.673946574	0.001121181
IQSEC2	*RNF216*	0.653879589	0.001765017
IQSEC2	*RNPS1*	0.674196535	0.001114626
IQSEC2	*RNUXA*	0.659229729	0.00156888
IQSEC2	*ROCK2*	0.627719556	0.003045161
IQSEC2	*RPS6KB2*	0.697266649	0.000633096
IQSEC2	*RPUSD1*	0.778677211	5.25E-05
IQSEC2	*RRP9*	0.71486076	0.000396936
IQSEC2	*SAE1*	0.641874631	0.002280859
IQSEC2	*SBF1*	0.605445159	0.004671712
IQSEC2	*SCAF1*	0.685392576	0.000852304
IQSEC2	*SCN3B*	0.605378533	0.00467748
IQSEC2	*SDC3*	0.645246598	0.00212467
IQSEC2	*SEC14L2*	0.665472335	0.001363472
IQSEC2	*SEMA6B*	0.756214685	0.000114505
IQSEC2	*SEZ6*	0.661922372	0.001477306
IQSEC2	*SFMBT1*	0.669558439	0.001241663
IQSEC2	*SH2D3C*	0.662426703	0.001460664
IQSEC2	*SHC3*	0.665306959	0.001368606

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*SHE*	-0.651058641	0.001876445
IQSEC2	*SIL1*	0.610968373	0.004213538
IQSEC2	*SIRT7*	0.614463989	0.003943259
IQSEC2	*SLC12A8*	0.600710053	0.005096557
IQSEC2	*SLC1A2*	0.645950207	0.002093233
IQSEC2	*SLC1A6*	0.669186356	0.001252362
IQSEC2	*SLC25A22*	0.686072831	0.000838214
IQSEC2	*SLC25A23*	0.718794648	0.000355943
IQSEC2	*SLC25A42*	0.697873891	0.000623312
IQSEC2	*SLC2A6*	0.607187045	0.00452298
IQSEC2	*SLC35E1*	0.703860283	0.000533518
IQSEC2	*SLC3A2*	0.636894692	0.002528988
IQSEC2	*SLC4A3*	0.693343323	0.000699503
IQSEC2	*SLC9A10*	-0.654817642	0.001729216
IQSEC2	*SMCR7*	0.671328253	0.001191822
IQSEC2	*SMPD1*	0.612676352	0.004079625
IQSEC2	*SMURF2*	0.612074383	0.004126414
IQSEC2	*SNAPC1*	0.62275679	0.003359026
IQSEC2	*SNRPA*	0.690509049	0.000751065
IQSEC2	*SNW1*	-0.666281763	0.001338576
IQSEC2	*ST3GAL2*	0.695265189	0.000666272
IQSEC2	*ST8SIA5*	0.734513907	0.000226064
IQSEC2	*STARD10*	0.64920733	0.001952727
IQSEC2	*STARD3*	0.644916611	0.002139549
IQSEC2	*STOML1*	0.602334428	0.004947367
IQSEC2	*STUB1*	0.644423861	0.002161929
IQSEC2	*STX10*	0.656012456	0.001684498
IQSEC2	*SULT1A1*	0.661085723	0.001505266
IQSEC2	*SV2A*	0.635247609	0.002615849
IQSEC2	*SYT17*	0.740920203	0.000186202
IQSEC2	*SYT6*	0.617066663	0.003751485
IQSEC2	*SYT7*	0.719945529	0.000344655
IQSEC2	*SYT8*	0.630774991	0.002864348

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*TACSTD1*	0.672190368	0.00116816
IQSEC2	*TARS2*	0.686515027	0.00082916
IQSEC2	*TAS2R7*	-0.614397452	0.003948266
IQSEC2	*TATDN1*	-0.643102365	0.002222917
IQSEC2	*TCEAL5*	0.608171097	0.004440699
IQSEC2	*TCP11L1*	0.748968078	0.000144778
IQSEC2	*TEP1*	0.611789033	0.004148748
IQSEC2	*THAP4*	0.620969223	0.00347846
IQSEC2	*THAP7*	0.641872445	0.002280964
IQSEC2	*THG1L*	0.671990156	0.00117362
IQSEC2	*THRA*	0.655470397	0.001704664
IQSEC2	*TIMM44*	0.605764979	0.004644106
IQSEC2	*TLR9*	0.666663194	0.001326977
IQSEC2	*TMEM132A*	0.745979299	0.000159128
IQSEC2	*TMEM132D*	0.677798797	0.001023683
IQSEC2	*TMEM141*	0.695494827	0.000662392
IQSEC2	*TMEM161A*	0.647922244	0.002007189
IQSEC2	*TMEM175*	0.687765322	0.000804008
IQSEC2	*TMEM177*	0.617295999	0.003734964
IQSEC2	*TMEM184B*	0.622521542	0.003374547
IQSEC2	*TMEM19*	0.697968456	0.0006218
IQSEC2	*TMEM24*	0.721891086	0.000326266
IQSEC2	*TMEM59L*	0.704569563	0.000523647
IQSEC2	*TMEM82*	-0.603544679	0.00483857
IQSEC2	*TMUB2*	0.668518602	0.001271756
IQSEC2	*TNFAIP8L1*	0.653945495	0.001762482
IQSEC2	*TNFRSF14*	0.613293469	0.004032115
IQSEC2	*TOMM34*	0.615027842	0.003901038
IQSEC2	*TOP3B*	0.605372554	0.004677998
IQSEC2	*TP53I11*	0.740264418	0.000189984
IQSEC2	*TRAF1*	-0.629513473	0.002937887
IQSEC2	*TRIB2*	0.66194054	0.001476704
IQSEC2	*TRRAP*	0.61984277	0.003555517

(*Continued*)

Table 2 (Continued).

Query	Gene	cor	pvalue
IQSEC2	*TSPAN17*	0.705784583	0.000507098
IQSEC2	*TSSC4*	0.64301686	0.002226912
IQSEC2	*TTC16*	0.630620521	0.00287327
IQSEC2	*TTC29*	-0.683861538	0.000884751
IQSEC2	*TTLL1*	0.692944532	0.000706571
IQSEC2	*TXNRD2*	0.707150509	0.000489025
IQSEC2	*UBAC2*	0.603047258	0.004883045
IQSEC2	*UBB*	0.673573709	0.00113102
IQSEC2	*UBE2J2*	0.683952107	0.000882803
IQSEC2	*UBE2M*	0.662080997	0.001472054
IQSEC2	*UBXD1*	0.618934609	0.003618668
IQSEC2	*ULK1*	0.663025245	0.001441117
IQSEC2	*USP13*	0.614455741	0.003943879
IQSEC2	*USP20*	0.649101646	0.001957158
IQSEC2	*USP48*	-0.71498917	0.000395537
IQSEC2	*USP5*	0.616899152	0.00376359
IQSEC2	*WBSCR17*	0.631600483	0.002817062
IQSEC2	*WDR23*	0.64049771	0.002347338
IQSEC2	*WDR6*	0.62755799	0.00305498
IQSEC2	*WDR92*	0.603811702	0.004814833
IQSEC2	*WNT5B*	0.62189079	0.003416455
IQSEC2	*XKR4*	0.617249551	0.003738305
IQSEC2	*YIPF5*	0.6809455	0.000949441
IQSEC2	*ZBTB38*	0.601035086	0.005066411
IQSEC2	*ZBTB45*	0.659203304	0.001569802
IQSEC2	*ZMYND12*	0.602400071	0.004941415
IQSEC2	*ZNF264*	0.678029241	0.001018085
IQSEC2	*ZNF319*	-0.6609974	0.001508243
IQSEC2	*ZNF454*	0.658251452	0.001603309
IQSEC2	*ZNF560*	0.635984159	0.002576704
IQSEC2	*ZNF641*	0.649012257	0.001960913
IQSEC2	*ZNRF2*	-0.603044552	0.004883288
IQSEC2	*ZSCAN21*	-0.713302452	0.000414251

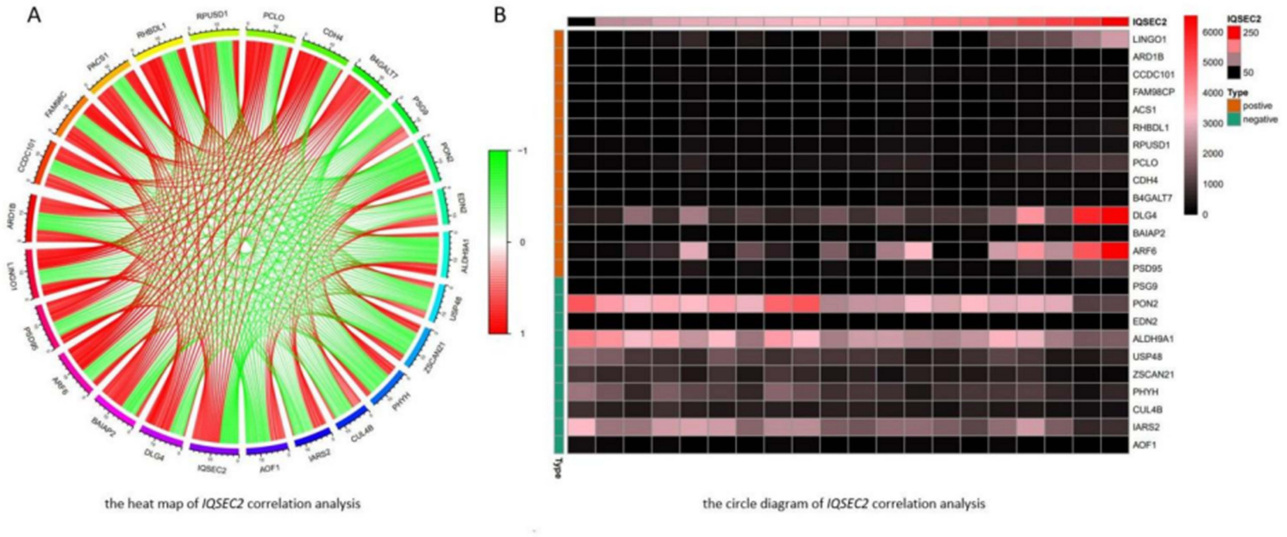

Figure 4 (**A**) Analysis circle diagram of Top 25 *IQSEC2* related genes, and approaching red indicates positive correlation, approaching green indicates negative correlation. (**B**) The heatmap of *IQSEC2* correlation analysis, and brown represents positive, green represents negative.

The Transcription Level of IQSEC2 and Related Genes Expression Affected by It

The expression levels of *IQSEC2* in probands 1 and 2 were significantly lower than those in their core families and control group (P <0.01) (Figure 3B), and so were those of *PSD-95* and *SAP97*, which were not observed in their parents (P <0.01) (Figure 5A and B). Conversely, the expression level of ARF6 in the two probands was significantly higher than that in their parents and normal control group (Figure 5C). However, the expression level of *IRSP53* in proband 1 was

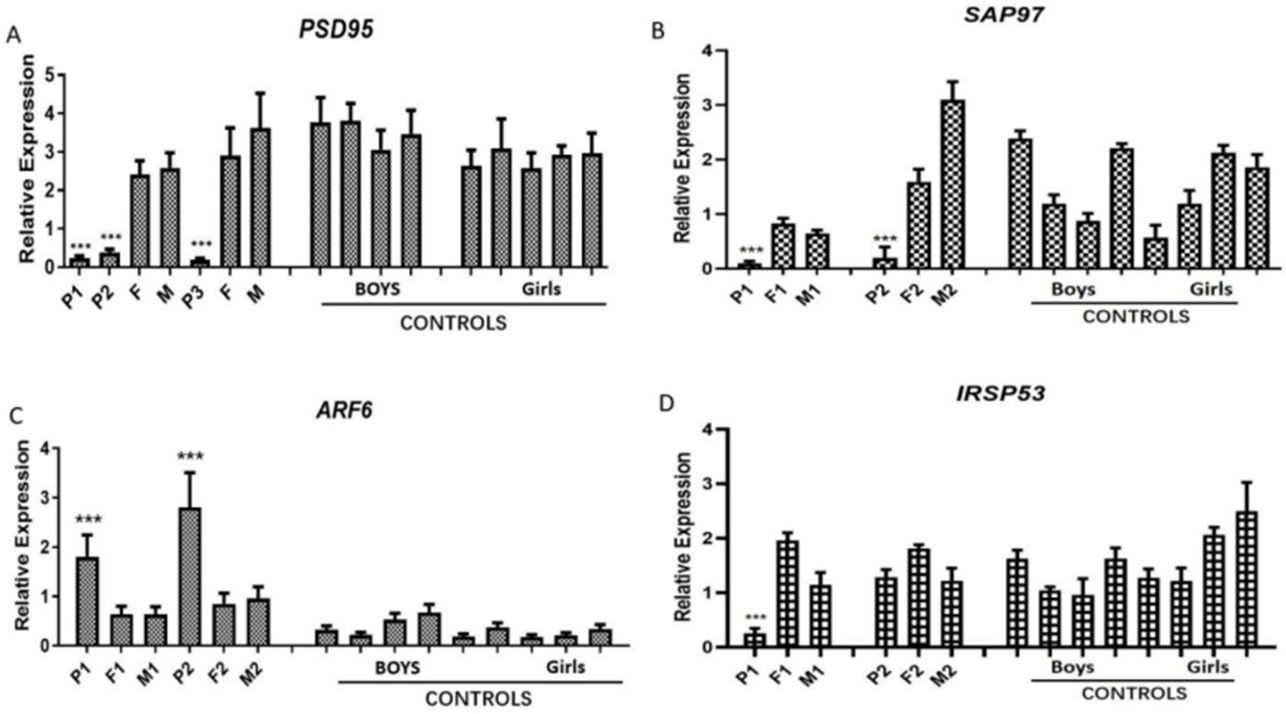

Figure 5 (**A**) The expression levels of *PSD-95* in probands 1 and 2 were significantly lower than normal levels, which were not observed in their parents. (**B**) The expression levels of *SAP97* in probands 1 and 2 were significantly lower than normal levels, which were not observed in their parents. (**C**) The expression levels of *ARF6* in probands 1 and 2 were significantly higher than those in their parents and normal control group. (**D**) The expression level of *IRSP53* in proband 1 was significantly lower than that in the parents and control group, while there was no significant difference in the expression level of *IRSP53* in proband 2 compared with their parents and control group.

significantly lower than that in the parents and control group, while there was no significant difference in the expression level of *IRSP53* in proband 2 compared with their parents and control group (P <0.01) (Figure 5D).

Changes in the Protein Structure and Function

We compared the three-dimensional structures of the *IQSEC2* wild-type (Figure 6A and B) and the nonsense (Figure 6C) and missense (Figure 6D and E) variants. The three-dimensional structures of the two mutants differed from those of the wild-type. We speculate that these differences may change the structure of the protein, thus affecting its function and stability.

Prediction of RNA Molecular Structure

We compared the RNA secondary structure of wild-type *IQSEC2* with that of missense mutation. The secondary structure of wild-type RNA (Figure 2C) was different from that of missense mutant RNA (Figure 2D). We speculate that the change in the structure of the missense mutant RNA may lead to its instability and degradation before it is translated into protein. Therefore, the expression of *IQSEC2* of missense mutation in proband 2 was reduced.

Conservation of the Missense and Nonsense Mutations

Conservation studies were conducted for c.3576C>A (p. Tyr1192*) and c.2983C>T (p. Arg995Trp) in humans, *Canis lupus familiaris, Mus musculus, Rattus norvegicus, Bos taurus, Xenopus tropicalis*, and *Danio rerio* (Figure 7A). These analyses suggest that the mutations are relatively stable.

Pathogenicity of the Genetic Variants

We evaluated the pathogenicity of the identified nonsense [c. 3576C > A (p.Tyr1192*)] and missense [c.2983C>T (p. Arg995Trp)] mutations using various pathogenicity prediction programs. The results indicate that the two mutants were

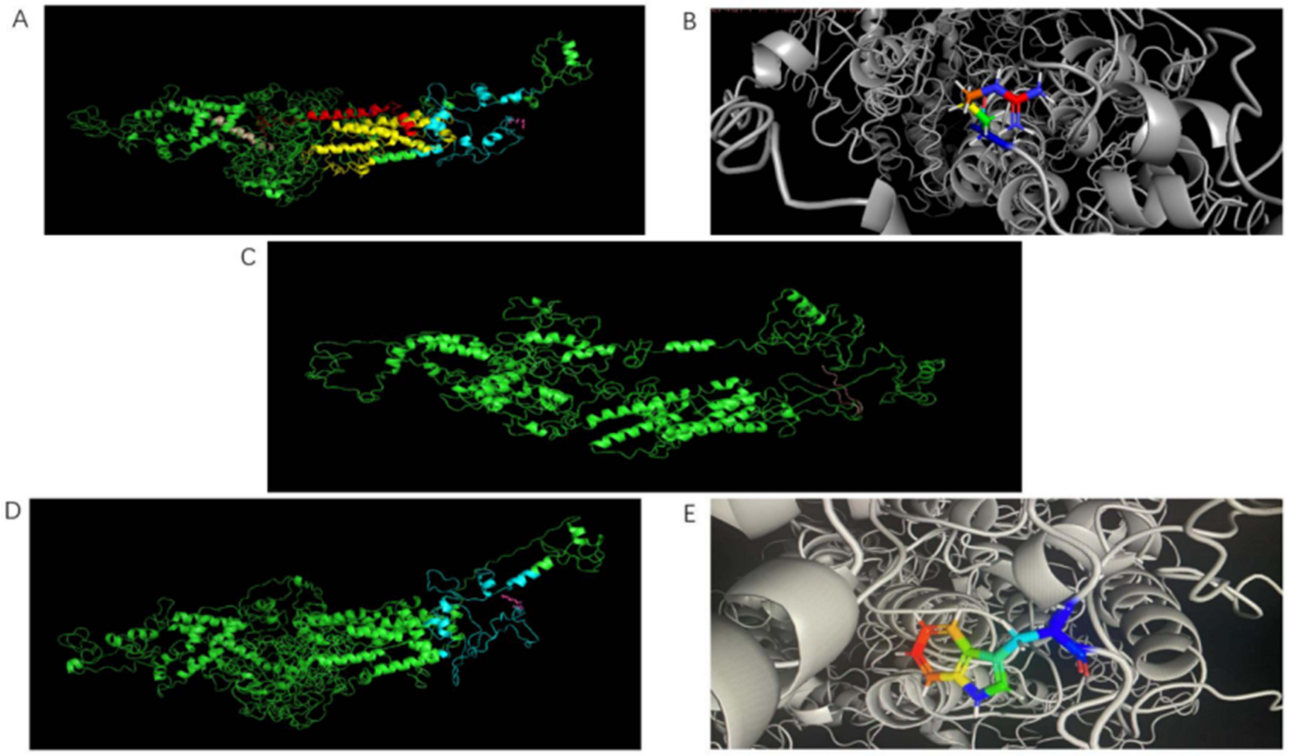

Figure 6 (**A**) Three-dimensional structure of *IQSEC2* wild-type protein. (**B**) Fine structure of wild-type amino acid at position 1192. (**C**) Nonsense mutation c.3576C>A (p. Tyr1192*). (**D**) Missense mutation c.2983C>T (p. Arg995Trp). (**E**) Fine structure of missense mutation at position 995.

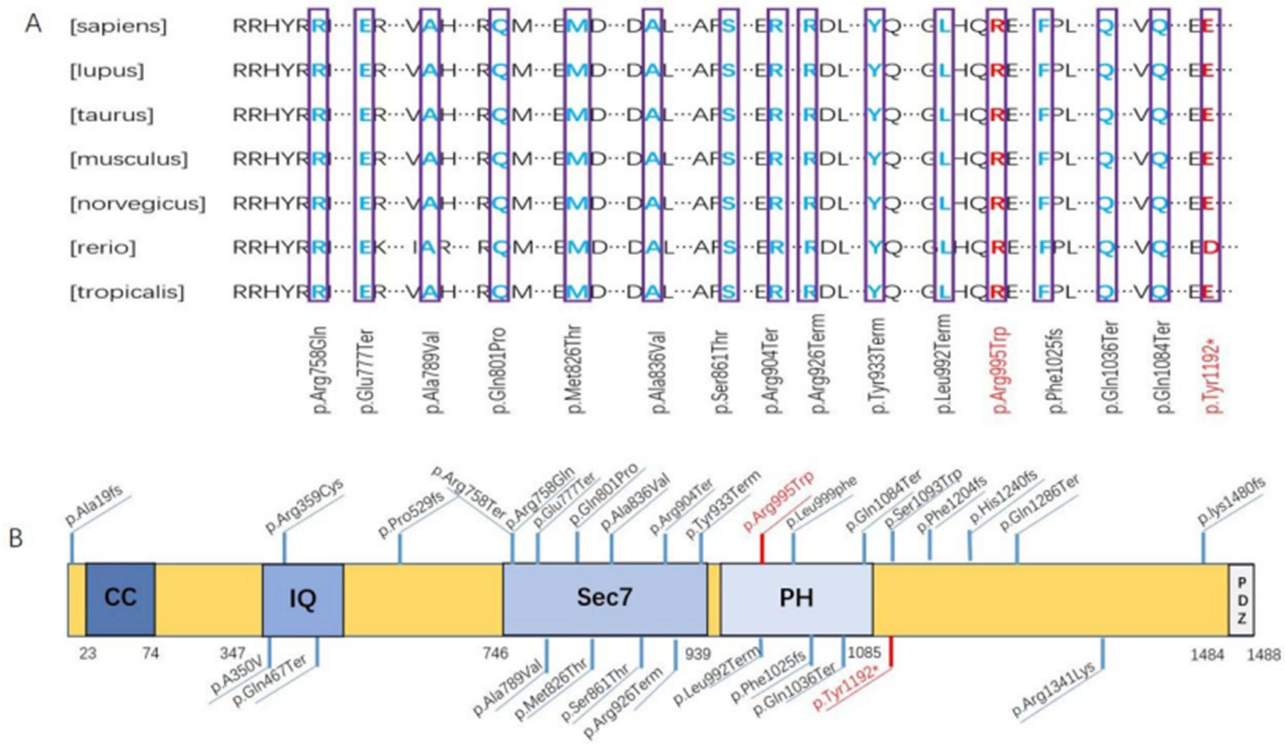

Figure 7 (**A**) The IQSEC2 protein sequence. Conservative studies were conducted for c.3576C>A (p. Tyr1192*) and c.2983C>T (p. Arg995Trp) in humans, *Canis lupus familiaris, Mus musculus, Rattus norvegicus, Bos Taurus, Xenopus tropicalis*, and *Danio rerio*. (**B**) Primary structure of *IQSEC2* and the common mutation sites. Coiled-coil domain (CC): 23–74; IQ motif (IQ): 347–376; SEC7 domain (SEC7):746–939; pleckstrin homology (PH): 951–1085; PDZ-binding motif (PDZ):1484–1488.

potentially harmful (SIFT score <0.05; PROVEAN score <-2.5; PolyPhen-2 score = 1; Mutation-Taster: Phylop > 1, Phastcons = 1). According to ACMG standard guidelines, the two variants were classified as likely pathogenic.

Neurological Function Test Scale Results

For proband 1, the WSC-MBD was positive, indicating developmental deviation. The ABC results were as follows: perception ability 30, communication ability 34, sports ability 38, language ability 31, and self-care ability 25, resulting in a total score of 158 (standard value = 53). Meanwhile, for proband 2, the WSC-MBD was positive, indicating developmental deviation. The ABC results were as follows: perception ability 30, communication ability 32, sports ability 35, language ability 30, and self-care ability 25, resulting in a total score of 152 (standard value = 53). For both probands, as the values obtained indicated the possibility of autism, the S-M score was determined. For both, the score was 5 points, lower than the standard value of 10 points. These scores indicate that the social life skills of both patients were lower than those corresponding to the normal value.

Discussion

Herein, we reported two mutants of the *IQSEC2* in two male children, one novel nonsense mutation [c. 3576C>A (p. Tyr1192*)] (*de novo*) and one missense mutation [c.2983C>T p.Arg995Trp], which was previously detected in a female patient,[4] has now been identified for the first time in a male patient. The clinical manifestations in the two children were consistent with the disease characteristics of *IQSEC2* mutations. Both mutants were hemizygous, and none are included in the normal population database gnome AD.

We speculate that the new nonsense mutation c.3576C>A (p. Tyr1192*) found in proband 1 may lead to the clinical features such as intellectual development disorder and epilepsy. There are several reasons: 1. The nonsense mutation identified in proband 1 will cause terminated the translation of its encoded protein at Tyr1192, resulting in the formation of truncated protein. Compared with the wild-type protein, the mutated protein will miss 297 amino acids at the

C-terminus (Figure 6C). From the domain of *IQSEC2*, we found that the missing amino acids caused *IQSEC2* to lose its PDZ binding motif. *IQSEC2* is located in the PSD of excitatory synapses through the interaction between its C-terminal PDZ-binding motif and *PSD-95*.[5] The PDZ-binding motif of *IQSEC2* maintains basal synaptic transmission as well as participates in *AMPAR* activity-dependent clearance.[2] However, mutations in the PDZ domain of *IQSEC2* destroy the regulation and neurotransmission of glutamate receptors in hippocampal cultures[2] and alter the localization of *IQSEC2* on dendritic spines.[6] This suggests that the nonsense mutation lacking PDZ binding motif (the key functional domain at the C-terminal) will seriously affect the function and structure of IQSEC2 protein. 2. We predicted the three-dimensional protein structure of mutant and wild-type. Research has found that truncated proteins are significantly different from proteins in the wild-type (Figure 6A-C). 3. We conducted further research on the mutants, after conducting RT qPCR analysis on their cDNA, the results showed that the significantly reduced expression level of *IQSEC2* in patients compared with that in their parents, brothers (Figure 3B). We speculate that owing to the early termination codon, the mRNA most likely undergoes nonsense mediated RNA decay (NMD), thereby avoiding generation of a truncated protein that may be harmful to cells.[7]

The missense mutation c.2983C>T (p.Arg995Trp) identified in proband 2, which results in an arginine-to-tryptophan substitution at amino acid 995, Although the missense mutation found in proband 2 has been reported, no relevant experiments have been conducted.[4] In this report, we conducted some research and found that the mutant may be the pathogenic gene of the patient, for the following reasons: 1. Conservative research has found that this site is in a conserved position in biological evolution, and mutations may alter its function (Figure 7A). 2. Additionally, by predicting the three-dimensional structure of the missense mutation, we observed that the protein structure of the mutant significantly differed from that of the wild-type (Figure 6D and E). The mutation site is located in the pleckstrin homology domain (Figure 7B), which is the primary membrane-bounding domain in the human protein. It combines with phosphoinositide (PI) at different affinities and specificities and plays a vital role in membrane transport and membrane localization.[8,9] ARF-GTPase-activating protein (ARF-GAP) is a key regulator of intracellular membrane transport, which is activated by Arf-GEF. Conversely, *IQSEC2*, which belongs to the GEF protein family and has a complete PH domain, can enhance the exchange between GTP and GDP, thus activating ARFs more effectively.[10] Therefore, we speculated that the mutation site in the PH domain might affect the coupling of *IQSEC2* Arf-GEF with membrane binding function,[9] thereby affecting the *IQSEC2* signaling pathway. 3. Real time quantitative PCR research has found a decrease in its expression level, indicating that it may lead to insufficient translation proteins, thereby affecting the stability of RNA. Therefore, the software (RNA fold web server) was used to predict the structure of wild-type *IQSEC2* RNA molecules and mutant RNA molecules; structural differences between the two were found. The change in the molecular structure of missense mutant RNA may make RNA unstable before it is translated into protein, thus degrading it.

We found a decrease in gene expression in both patients, which may be the cause of the disease. We suggest that if a mutation similar to this gene is found in the future, conducting expression testing may help determine whether the mutation is pathogenic.

Approximately 2% of patients with ID and epilepsy exhibit *IQSEC2* mutations.[11] The Human Gene Mutation Database (HGMD) includes 89 reported cases of *IQSEC2* mutations across 113 mutation sites. Among these, the most common is the frameshift mutation, which has been reported in 27 cases. The most common mutations among these are C.804 del C del 1 bp codon 268, which has been reported in 5 cases,[12–16] and c.2052 _ 2053 del CG del 2bp Codon 684 and c.4039dupG ins 1 bp codon 1347, which have been reported in 2 cases.[12,17] There are 27 nonsense mutations, among which the most common are [c.3163C>T(p.Arg1055*)] and [c.2563C>T(p.Arg855*)],[14,16,18] each of which has been reported in 3 cases and [c.2776C>T(p.Arg926*)] and [c.2317C>T(p.Gln773*)] and [c.895C>T(p. Gln299Term)], each of which has been reported in 2 cases.[14–16] There are 25 missense mutations, among which the most common are [[c.2312G>A (p.Gly771Asp)], [c.1049C>T(p.Ala350Val)], and [c.2582G>C(p.Ser861Thr)] which have been reported in 3 cases,[17,19–21] and [c.2507C>T(p.Ala836Val)] and [c.3206G>C(p.Arg1069Pro)] which have been reported in 2 cases.[15,22,23] There are 5 cases of splice, and intraframe mutations occurred in 3 cases[16,24,25] (Tables 3 and 4).We found that 61% (31/52) of these missense mutations and nonsense mutations were distributed in the SEC7 and PH region, and that these mutations were located in conserved sequences and important domains of the protein (Figure 7A and B),

Table 3 *IQSEC2* mutations previously reported

Mutation Type	Number of Times
Frameshift	27
Missense	25
Nonsense	27
Splice	5
Inframe	3

suggesting that the SEC7 and PH region plays an important role in the function of the IQSEC2 protein. And out of 113 reported mutation cases, 39% (44/113) were accompanied by epilepsy.

Mutations in *IQSEC2*, an X-linked gene, are associated with intellectual disability (ID), epilepsy and autism.[20,26] Four missense mutants of *IQSEC2* were identified in families diagnosed with X chromosome-linked intellectual disability (XLID).[27] Common clinical features and signs related to *IQSEC2* include language regression, stereotypic hand movements, hypotonia, ataxia, microcephaly, and seizures.[28] The common types of epilepsy associated with *IQSEC2* mutations include muscle tone disorders, myoclonus, and spasmodic epilepsy, and most of them are generalized epileptic seizures. However, in patients with combined ID, other symptoms include developmental delay, speech impairment, walking delay, and autism. Other rare symptoms may also include limited hand movement, strabismus, and low intraocular pressure.[20] In addition, research reports have found that differences were observed between males and females in patients with missense functional *IQSEC2* variants, with males demonstrating mild to severe non-syndromic ID accompanied by seizures and females with missense functional *IQSEC2* mutations being generally either asymptomatic carriers or mildly affected.[29] The research has shown that the majority of pathogenic variants are predicted to lead to premature termination that is subject to the RNA surveillance process of nonsense-mediated decay, leading to a complete loss of mutant IQSEC2 protein, male patients with loss of function variants invariably present with severe ID, seizures.[17] The two variants we reported highly conform to the clinical phenotype of *IQSEC2* pathogenesis. In previous reports, Subtelomere FISH analysis was performed on peripheral blood lymphocytes to investigate the pathogenicity of the X chromosome homozygous deletion 4q35.2 in *IQSEC2*.[30] However, research on *IQSEC2* A350V is mostly focused on mouse models, which investigate the pathogenicity of mutations by observing the behavior of mice and the expression of iqsec2 in the brain.[31–33] Our report is the first to explore the pathogenic mechanism of *IQSEC2* by examining its expression in peripheral blood cells of patients.

Through the analysis of the co expression of *IQSEC2* and the *IQSEC2* signaling pathway, four genes with significant expression of *IQSEC2* were screened *IQSEC2: PSD-95, SAP-97, ARF-6* and *IRSP 53*. To investigate whether the mutation of this gene affects the function of its downstream genes, we used quantitative PCR to further investigate the expression of *IQSEC2* related genes in patient blood samples. *IQSEC2* encodes IQSEC2 protein, which is an upstream regulatory factor of *PSD-95*. It can regulate the transcription and translation of *PSD-95* by interacting with *PSD-95* through PDZ binding motif. A lack of *PSD-95* results in impaired cognition and learning.[34] *PSD-95* exists in the 1.5 MDa NMDA complex, which also includes *IQSEC2*.In mice lacking *PSD-95*, IQSEC2 could not form the above complex.[35] We observed that the expression of *PSD-95* in probands 1 and 2 was significantly lower than in the control group and the parents of proband 1 (Figure 5A). We speculate that the mutation of *IQSEC2* may affect the expression and function of IQSEC2 protein, leading to changes in the expression level of *PSD-95*.[36]

Petersen et al also found that *IQSEC2* also interacts with synapse associated protein 97 (SAP97) through PDZ. Research showed that deletion of the *SAP97* may lead to disrupted glutamate neurotransmitter transmission and impaired function of glutamate receptors.[37] We also found that IQSEC2 is a membrane protein that participates in vesicular transport[3] and binds with *SAP97* to participate in vesicle formation and play an important role in vesicular transport.[38] Therefore, we speculate that the decreased expression of *IQSEC2* in the proband 1 and

Table 4 Most common *IQSEC2* mutations and related phenotypes reported for research

Genetic Variations in *IQSEC2*							
PMID	**Gene**	**Variation (cDNA) NM_001111125.2**	**Variation (Protein) NP_001104595.1**	**Location**	**Annotation**	**Clinical Significance**	**Related Disorder**
26795593;25356970;29100083; 28815955;30206421	*IQSEC2*	c.804delC	del 1 bp codon 268	NA	deletion	Pathogenic	Epilepsy; Mental retardation, X-linked
24306141;26795593	*IQSEC2*	c.2052_2053delCG	del 2 bp codon 684	NA	deletion	Pathogenic	Intellectual disability; Epilepsy
27864847;30206421	*IQSEC2*	c.4039dupG	ins 1 bp codon 1347	NA	duplication	Likely pathogenic	Intellectual disability and epilepsy
27665735;30842726; 29026562	*IQSEC2*	c.1049C>T	A350V	IQ motif, EF-hand binding site	substution	Pathogenic	Intellectual disability, nonsyndromic
27652284;30255931; 30206421	*IQSEC2*	c.2312G>A	G771D	Sec7 domain	substitution	Pathogenic	Intellectual disability and epilepsy
24306141;25356970; 26795593	*IQSEC2*	c.2582G>C	S861T	Sec7 domain|Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability and Epilepsy
28815955;3066632	*IQSEC2*	c.2507C>T	A836V	Sec7 domain|Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability, syndromic
29720203;30206421	*IQSEC2*	c.3206G>C	R1069P	IQ motif and SEC7 domain-containing protein, PH domain| PH domain-like|Pleckstrin homology domain;Sec7 domain| Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability
25649377;29100083; 30206421	*IQSEC2*	c.3163C>T	R1055Term	IQ motif and SEC7 domain-containing protein, PH domain| PH domain-like|Pleckstrin homology domain;Sec7 domain| Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability
23020937;28191890; 23674175	*IQSEC2*	c.2563C>T	R855Term	Sec7 domain|Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability, nonsyndromic
27062609;30206421	*IQSEC2*	c.2776C>T	R926Term	Sec7 domain|Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Rett-like syndrome;
28815955;30206421	*IQSEC2*	c.2317C>T	Q773Term	Sec7 domain	substitution	Likely pathogenic	Intellectual disability, syndromic
29100083;30206421	*IQSEC2*	c.895C>T	Q299Term	NA	substitution	Uncertain significance	Developmental and epileptic encephalopathy

Abbreviations: R, Arginine; G, Glycine; Q, Glutamine; S, Serine; P, Proline; V, Valine; T, Threonine; A, Alanine; D, Aspartic acid.

proband 2 may affect the localization and stability of *SAP97*, resulting in a decrease in *SAP97* expression (Figure 5B). This impedes vesicle formation and transport, leading to the development of the disease.

Arf-6 protein is the substrate of *IQSEC2*-GEF. Although most mammals have six kinds of ARF proteins, only arf-6 is related to the transport of cell membrane substances and the regulation of neurotransmitters. *AFR6* is one of the downstream target *genes of IQSEC2*. Consequently, arf-6 is involved in the *IQSEC2* signal transduction pathway.[5] *IQSEC2* promotes the exchange of GDP and GTP on the arf-6 protein, leading to its activation. arf-6 activation subsequently activates a downstream effector, which leads to changes in cell membrane transport and actin dynamics. These physiological processes are closely related to the development of cortical neurons, formation of dendritic spines, and promotion of prominent plasticity[39] speculated that the over-expression of ARF-6 delays neuronal migration. *ARF6* mRNA expression in probands 1 and 2 was significantly higher than that in their parents and control group (Figure 5C). The results indicate that the overexpression of *ARF6* caused by *IQSEC2* mutations may be related to the pathogenesis of epilepsy and ID. Unfortunately, we did not obtain sufficient sample size. Our experiment had some shortcomings, such as the activity changes of *ARF6*-GEF. We intend to further verify the role of *ARF6* activity changes caused by *IQSEC2* mutations in ID and epilepsy in future studies.

Previous studies have demonstrated that the interaction between *IQSEC2* and *IRSp53* is mediated by a proline-rich sequence (aa 1424–1434) located in the C-terminus.[33] *IRSp53* has been shown to regulate spine formation downstream of the Rac1 pathway.[40] Meanwhile, *ARF6* can regulate dendritic and spine formation upstream of Rac1.[41,42] The interaction between *IQSEC2* and *IRSp53* activates *ARF6* at the optimal position, and the activated *ARF6* can enhance Rac1 near its effector *IRSp53*. Therefore, the reduction of *IRSp53* caused by *IQSEC2* mutation may hinder the formation and maintenance of dendrites and spines. Our experiment showed that the expression level of *IRSP53* in proband 1 was lower than that of the parents and control group, but there was no change in the expression level of proband 2 (Figure 5D). This result suggests that the nonsense mutation in proband 1 resulted in the loss of function of the truncated protein, which affected the expression level of *IRSP53*; while the missense mutation in proband 2 led to RNA instability, with the translated product may have retained some function, enabling it to interact with *IRSP53*, hence the expression level of *IRSP53* did not decrease.

The epilepsy caused by *IQSEC2* mutation has different clinical phenotypes.[9,20,43,44] The two patients in this study were diagnosed with IS, and the initial VEEG indicated peak rhythm disorders accompanied by frequent spasms. The EEG of infantile spasticity showed that high potential slow waves and spike slow complex waves are widely distributed, and low voltage activities are involved, which appear repeatedly and periodically. It is reported that periodic discharges will occur in the cortex, such as the inhibitory burst mode of nonketotic hyperglycinemia, and excessive N-methyl-D-aspartate (*NMDA*) transmission may cause these discharges.[45] The specific bidirectional modulation of *IQSEC2* on synaptic transmission is specific in the modulation activity dependent signaling process through *NMDA*.[2] Therefore, Patients with pathogenic variation of *IQSEC2* may have reduced synaptic transmission, so they show enhanced transmission, which is the basis of these characteristic periodic discharges on EEG. However, according to reports,[46] changes in glutamate receptors in brain regions should be considered a potential factor in the pathogenesis of infantile spasms. We speculate that the two variants we reported may have strong potential to affect *NMDA* receptors. This preliminary speculation needs further research and demonstration.

Conclusions

Our findings suggest that the two *IQSEC2* variants, including the *de novo* mutation, are likely to be highly pathogenic, and therefore provide novel insights into *IQSEC2* variant spectrum. In practice, clinical doctors can conduct early genetic testing and routine follow-up for children with growth retardation and epilepsy. And we also need to prevent heart and kidney diseases caused by mutations in this gene. We believe that a better understanding of the diseases associated with this gene mutation may lead to the development of novel diagnostics and mutation-specific personalized pharmacotherapy. In the future, we intend to obtain stem cells from affected patients to further explore the possible mutation-specific functional alterations to clarify the function of *IQSEC2*.

Study Limitations

Given that children are the most vulnerable population and their diseases are complex and diverse. The most important limitations of this study are the lack of new variants or the same variant from many infants, as well as the absence of

other tissue samples for validation. More tissue samples and mutants will be able to explore the pathogenic mechanisms of variants from different perspectives.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Shanghai Children's Hospital (Approval number: 2019R071-F03) and complies with the ethical principles of the Helsinki Declaration. And the authors have obtained informed consent from the patient's guardian regarding the publication of the article.The parents/guardians of both patients have agreed to publish case details and institutional approval was not required to publish case details.

Acknowledgments

We would like to express our gratitude to the patients and their parents for their cooperation. We would also like to thank Chao Wang for his help during this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by grants from the Shanghai Key Clinical Specialty Project (grant number shslczdzk05705), Scientific Research Fund of China Association Against Epilepsy (grant number CJ-A-2021-07); National Fund Cultivation Special Project of Shanghai Children's Hospital (grant number 2021YGZQ05) and Scientific Research Fund of China Association Against Epilepsy (CX-2022-013).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Levy NS, Umanah GKE, Rogers EJ, Jada R, Lache O, Levy AP. IQSEC2-Associated Intellectual Disability and Autism. *Int J Mol Sci*. 2019;20(12):12. doi:10.3390/ijms20123038
2. Brown JC, Petersen A, Zhong L, et al. Bidirectional regulation of synaptic transmission by BRAG1/IQSEC2 and its requirement in long-term depression. *Nat Commun*. 2016;7:11080. doi:10.1038/ncomms11080
3. Ewans LJ, Field M, Zhu Y, et al. Gonadal mosaicism of a novel IQSEC2 variant causing female limited intellectual disability and epilepsy. *Eur J Hum Genet*. 2017;25(6):763–767. doi:10.1038/ejhg.2017.29
4. Srivastava S, Desai S, Cohen J, et al. Monogenic disorders that mimic the phenotype of Rett syndrome. *Neurogenetics*. 2018;19(1):41–47. doi:10.1007/s10048-017-0535-3
5. Sakagami H, Sanda M, Fukaya M, et al. IQ-ArfGEF/BRAG1 is a guanine nucleotide exchange factor for Arf6 that interacts with PSD-95 at postsynaptic density of excitatory synapses. *Neurosci Res*. 2008;60(2):199–212. doi:10.1016/j.neures.2007.10.013
6. Kang J, Park H, Kim E. IRSp53/BAIAP2 in dendritic spine development, NMDA receptor regulation, and psychiatric disorders. *Neuropharmacology*. 2016;100:27–39. doi:10.1016/j.neuropharm.2015.06.019
7. Oren YS, Pranke IM, Kerem B, Sermet-Gaudelus I. The suppression of premature termination codons and the repair of splicing mutations in CFTR. *Curr Opin Pharmacol*. 2017;34:125–131. doi:10.1016/j.coph.2017.09.017
8. Feng J, He L, Li Y, Xiao F, Hu G. Modeling of PH Domains and Phosphoinositides Interactions and Beyond. *Protein Reviews*. 2018;19–32.
9. Shoubridge C, Dudding-Byth T, Pasquier L, Goel H, Yap P, McConnell V. IQSEC2-related encephalopathy in males due to missense variants in the pleckstrin homology domain. *Clin Genet*. 2022;102(1):72–77. doi:10.1111/cge.14136
10. Aizel K, Biou V, Navaza J, et al. Integrated conformational and lipid-sensing regulation of endosomal ArfGEF BRAG2. *PLoS Biol*. 2013;11(9):e1001652. doi:10.1371/journal.pbio.1001652
11. Heyne HO, Singh T, Stamberger H, et al. De novo variants in neurodevelopmental disorders with epilepsy. *Nat Genet*. 2018;50(7):1048–1053. doi:10.1038/s41588-018-0143-7
12. Helbig KL, Farwell Hagman KD, Shinde DN, et al. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genet Med*. 2016;18(9):898–905. doi:10.1038/gim.2015.186
13. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genet Med*. 2015;17(7):578–586. doi:10.1038/gim.2014.154

14. Hamdan FF, Myers CT, Cossette P, et al. High Rate of Recurrent De Novo Mutations in Developmental and Epileptic Encephalopathies. *Am J Hum Genet*. 2017;101(5):664–685. doi:10.1016/j.ajhg.2017.09.008
15. Helm BM, Powis Z, Prada CE, et al. The role of IQSEC2 in syndromic intellectual disability: narrowing the diagnostic odyssey. *Am J Med Genet A*. 2017;173(10):2814–2820. doi:10.1002/ajmg.a.38404
16. Mignot C, McMahon AC, Bar C, et al. IQSEC2-related encephalopathy in males and females: a comparative study including 37 novel patients. *Genet Med*. 2019;21(4):837–849. doi:10.1038/s41436-018-0268-1
17. Gandomi SK, Farwell Gonzalez KD, Parra M, et al. Diagnostic exome sequencing identifies two novel IQSEC2 mutations associated with X-linked intellectual disability with seizures: implications for genetic counseling and clinical diagnosis. *J Genet Couns*. 2014;23(3):289–298. doi:10.1007/s10897-013-9671-6
18. Tzschach A, Grasshoff U, Beck-Woedl S, et al. Next-generation sequencing in X-linked intellectual disability. *Eur J Hum Genet*. 2015;23(11):1513–1518. doi:10.1038/ejhg.2015.5
19. de Kovel CG, Brilstra EH, van Kempen MJ, et al. Targeted sequencing of 351 candidate genes for epileptic encephalopathy in a large cohort of patients. *Mol Genet Genomic Med*. 2016;4(5):568–580. doi:10.1002/mgg3.235
20. Zerem A, Haginoya K, Lev D, et al. The molecular and phenotypic spectrum of IQSEC2-related epilepsy. *Epilepsia*. 2016;57(11):1858–1869. doi:10.1111/epi.13560
21. Hynynen J, Pokka T, Komulainen-Ebrahim J, et al. Variants p.Q1236H and p.E1143G in mitochondrial DNA polymerase gamma POLG1 are not associated with increased risk for valproate-induced hepatotoxicity or pancreatic toxicity: a retrospective cohort study of patients with epilepsy. *Epilepsia*. 2018;59(11):2125–2136. doi:10.1111/epi.14568
22. Staněk D, Laššuthová P, Štěrbová K, et al. Detection rate of causal variants in severe childhood epilepsy is highest in patients with seizure onset within the first four weeks of life. *Orphanet J Rare Dis*. 2018;13(1):71. doi:10.1186/s13023-018-0812-8
23. Radley JA, O'Sullivan RBG, Turton SE, et al. Deep phenotyping of 14 new patients with IQSEC2 variants, including monozygotic twins of discordant phenotype. *Clin Genet*. 2019;95(4):496–506. doi:10.1111/cge.13507
24. Zhang Y, Kong W, Gao Y, et al. Gene Mutation Analysis in 253 Chinese Children with Unexplained Epilepsy and Intellectual/Developmental Disabilities. *PLoS One*. 2015;10(11):e0141782. doi:10.1371/journal.pone.0141782
25. Sajan SA, Jhangiani SN, Muzny DM, et al. Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in MECP2. *Genet Med*. 2017;19(1):13–19. doi:10.1038/gim.2016.42
26. Shoubridge C, Harvey RJ, Dudding-Byth T. IQSEC2 mutation update and review of the female-specific phenotype spectrum including intellectual disability and epilepsy. *Hum Mutat*. 2019;40(1):5–24. doi:10.1002/humu.23670
27. Shoubridge C, Tarpey PS, Abidi F, et al. Mutations in the guanine nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. *Nat Genet*. 2010;42(6):486–488. doi:10.1038/ng.588
28. Alexander-Bloch AF, McDougle CJ, Ullman Z, Sweetser DA. IQSEC2 and X-linked syndromal intellectual disability. *Psychiatr Genet*. 2016;26(3):101–108. doi:10.1097/ypg.0000000000000128
29. Brant B, Stern T, Shekhidem HA, et al. IQSEC2 mutation associated with epilepsy, intellectual disability, and autism results in hyperexcitability of patient-derived neurons and deficient synaptic transmission. *Mol Psychiatry*. 2021;26(12):7498–7508. doi:10.1038/s41380-021-01281-0
30. Karaman Mercan T, Altiok Clark O, Erkal O, et al. Coexistence of a Homozygous Chromosome 4q35.2 Deletion and Hidden IQSEC2 Pathogenic Variant in a Child with Intellectual Disability. *Cytogenet Genome Res*. 2021;161(3–4):153–159. doi:10.1159/000515368
31. Lichtman D, Bergmann E, Kavushansky A, et al. Structural and functional brain-wide alterations in A350V Iqsec2 mutant mice displaying autistic-like behavior. *Transl Psychiatry*. 2021;11(1):181. doi:10.1038/s41398-021-01289-8
32. Myers KR, Wang G, Sheng Y, Conger KK, Casanova JE, Zhu JJ. Arf6-GEF BRAG1 regulates JNK-mediated synaptic removal of GluA1-containing AMPA receptors: a new mechanism for nonsyndromic X-linked mental disorder. *J Neurosci*. 2012;32(34):11716–11726. doi:10.1523/JNEUROSCI.1942-12.2012
33. Sanda M, Kamata A, Katsumata O, et al. The postsynaptic density protein, IQ-ArfGEF/BRAG1, can interact with IRSp53 through its proline-rich sequence. *Brain Res*. 2009;1251:7–15. doi:10.1016/j.brainres.2008.11.061
34. Coley AA, Gao WJ. PSD95: a synaptic protein implicated in schizophrenia or autism? *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;82:187–194. doi:10.1016/j.pnpbp.2017.11.016
35. Frank RAW, Zhu F, Komiyama NH, Grant SGN. Hierarchical organization and genetically separable subfamilies of PSD95 postsynaptic supercomplexes. *J Neurochem*. 2017;142(4):504–511. doi:10.1111/jnc.14056
36. Petersen A, Brown JC, Gerges NZ. BRAG1/IQSEC2 as a regulator of small GTPase-dependent trafficking. *Small GTPases*. 2020;11(1):1–7. doi:10.1080/21541248.2017.1361898
37. Sato J, Shimazu D, Yamamoto N, Nishikawa T. An association analysis of synapse-associated protein 97 (SAP97) gene in schizophrenia. *J Neural Transm*. 2008;115(9):1355–1365. doi:10.1007/s00702-008-0085-9
38. Zheng CY, Seabold GK, Horak M, Petralia RS. MAGUKs, synaptic development, and synaptic plasticity. *Neuroscientist*. 2011;17(5):493–512. doi:10.1177/1073858410386384
39. Falace A, Buhler E, Fadda M, et al. TBC1D24 regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. *Proc Natl Acad Sci U S A*. 2014;111(6):2337–2342. doi:10.1073/pnas.1316294111
40. Choi J, Ko J, Racz B, et al. Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases. *J Neurosci*. 2005;25(4):869–879. doi:10.1523/jneurosci.3212-04.2005
41. Choi S, Ko J, Lee JR, et al. ARF6 and EFA6A regulate the development and maintenance of dendritic spines. *J Neurosci*. 2006;26(18):4811–4819. doi:10.1523/jneurosci.4182-05.2006
42. Hernández-Deviez DJ, Casanova JE, Wilson JM. Regulation of dendritic development by the ARF exchange factor ARNO. *Nat Neurosci*. 2002;5(7):623–624. doi:10.1038/nn865
43. Izumi T, Aihara Y, Kikuchi A, Kure S. Electroencephalographic findings and genetic characterization of two brothers with IQSEC2 pathogenic variant. *Brain Dev*. 2021;43(5):652–656. doi:10.1016/j.braindev.2020.12.020
44. Choi MH, Yang JO, Min JS, et al. A Novel X-Linked Variant of IQSEC2 is Associated with Lennox-Gastaut Syndrome and Mild Intellectual Disability in Three Generations of a Korean Family. *Genet Test Mol Biomarkers*. 2020;24(1):54–58. doi:10.1089/gtmb.2019.0177

45. Dulac O. Epileptic encephalopathy with suppression-bursts and nonketotic hyperglycinemia. *Handb Clin Neurol*. 2013;113:1785–1797. doi:10.1016/b978-0-444-59565-2.00048-4
46. Riikonen R. Biochemical mechanisms in pathogenesis of infantile epileptic spasm syndrome. *Seizure*. 2023;105:1–9. doi:10.1016/j.seizure.2023.01.004