

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 Ca²⁺ influx, which in turn binds to

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 μ L of blood was collected, and total RNA was extracted using a Qiagen kit (Qiagen Inc., Germany) according to the manufacturer's instructions (Yeasten 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 μ L (\leq 500 ng); 5 \times gDNA digester mix, 3 μ L; RNase free ddH₂O, 10 μ L. The sample was centrifuged briefly (8000 ref, 5 min, 4 °C) and incubated at 42 °C for 2 min. Subsequently, 5 μ L of 4 \times 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 \geq 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 μ L) contained cDNA (2 μ L), forward primer F (10 μ M; 1 μ L), reverse primer R (10 μ M; 1 μ L), 2 \times SYBR Premix Ex Taq (10 μ L), and sterilized water (6 μ L).

The reaction conditions for qPCR were as follows: pre-denaturation at 95 $^{\circ}$ C for 30s, 40 cycles of denaturation at 95 $^{\circ}$ C for 5 s, and renaturation at 60 $^{\circ}$ C for 20s, followed by a final extension at 65 $^{\circ}$ C for 15s. The relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ 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.cmb.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'
<i>IQSEC2</i>	CCAGAAAGTGGAGCGACTCATC	GCAAGGATGAAGATGGTGTCTGG
<i>PSD-95</i>	TCCACTCTGACAGTGAGACCGA	CGTCACTGTCTCGTAGCTCAGA
<i>SAP97</i>	CGACCTGAAGAATACAGTCGT	GGGATCGCTTCTGGCTAGTTC
<i>ARF-6</i>	ATCTTCGCCAACAAAGCAGGA	AGGGCTGCACATACCAGTTC
<i>IRSP53</i>	ATGGAGCAGTTCAACCCTAGC	TTCACCAGGGCGTCAAAGTAG
<i>GAPDH</i>	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 $2^{-\Delta\Delta C_t}$ 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 $\mu\text{mol/L}$ 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

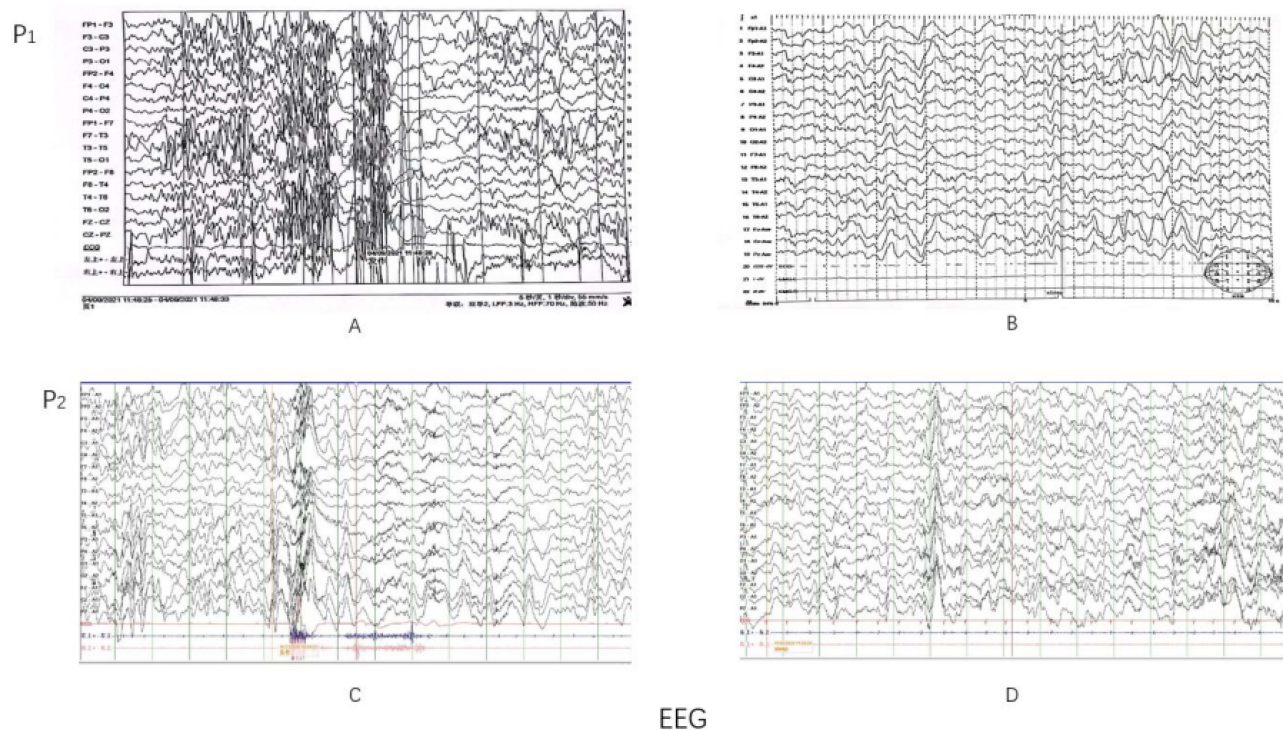


Figure 1 Electrocardiograms of patients (A) Proband 1 (P1), April 2021, VEEG: spasms seizure; (B) September 2021, VEEG: the seizure disappeared, and peak rhythm disorder disappeared; (C) Proband 2 (P2), October 2019, VEEG: spasms seizure; (D) August 2021, VEEG: After sleep, the rear head showed epileptiform 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_00111125.3) 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_00111125.3) c.2983C>T (p. Arg995Trp) hemizygote variation in exon 10 of *IQSEC2* in proband 2. This mutation resulted in an amino

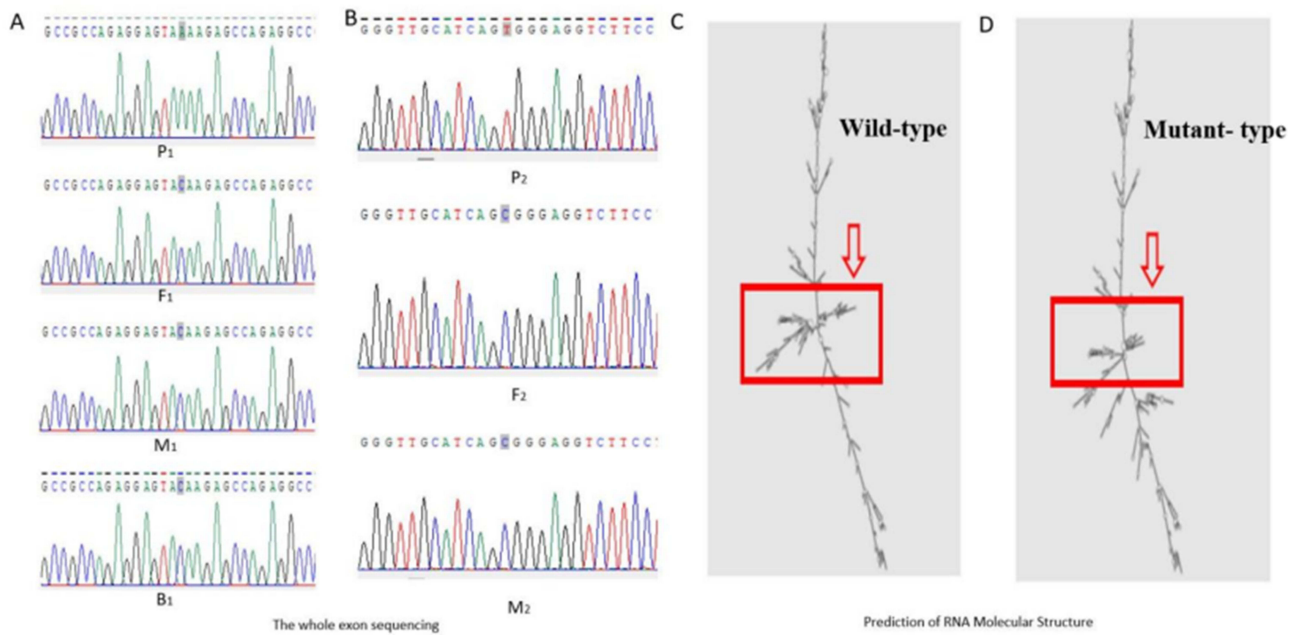


Figure 2 (A) The sequences of genomic DNA suggest that the nonsense mutation of *IQSEC2* c.3576C>A (p. Tyr1192*) was identified in proband 1 but not in his parents and brothers. (B) Missense mutation of *IQSEC2* c.2983C>T (p. Arg995Trp) in proband 2 but not in his parents. (C) Secondary molecular structures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

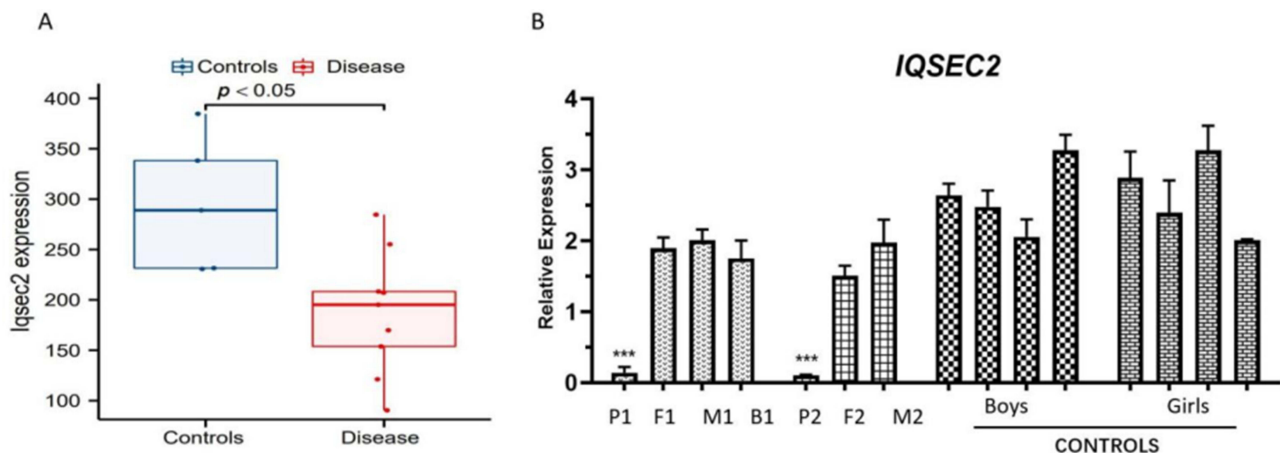


Figure 3 (A) Analysis of variance between *IQSEC2* and the normal group. The left vertical axis represents gene expression levels, the red box plot represents gene expression levels in the disease group, and the blue box plot represents gene expression levels in the control group. The results showed 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
<i>IQSEC2</i>	<i>ABCC5</i>	0.687554454	0.000808204
<i>IQSEC2</i>	<i>ABTB1</i>	0.6144283	0.003945944
<i>IQSEC2</i>	<i>ADAMTS2</i>	-0.603890044	0.004807887
<i>IQSEC2</i>	<i>ADCK5</i>	0.65310929	0.001794879
<i>IQSEC2</i>	<i>ADCY2</i>	0.633927858	0.002687229
<i>IQSEC2</i>	<i>ADCYAP1R1</i>	0.622605743	0.003368985
<i>IQSEC2</i>	<i>ADRA1D</i>	0.635031286	0.00262744
<i>IQSEC2</i>	<i>AGPAT2</i>	0.601183041	0.005052738
<i>IQSEC2</i>	<i>AHDC1</i>	0.620157205	0.003533867
<i>IQSEC2</i>	<i>AKAP3</i>	0.719575256	0.000348253
<i>IQSEC2</i>	<i>AKR7A2</i>	-0.614105161	0.003970324
<i>IQSEC2</i>	<i>ALDH9A1</i>	-0.722911702	0.000316958
<i>IQSEC2</i>	<i>ALKBH6</i>	0.717579003	0.000368206
<i>IQSEC2</i>	<i>ALKBH7</i>	0.68893181	0.000781126
<i>IQSEC2</i>	<i>ALKBH8</i>	0.634916268	0.00263362
<i>IQSEC2</i>	<i>ANKRD34</i>	0.673046723	0.00114505
<i>IQSEC2</i>	<i>ANKRD39</i>	0.629435324	0.002942494
<i>IQSEC2</i>	<i>ANTXR1</i>	-0.659610035	0.001555665
<i>IQSEC2</i>	<i>ANXA6</i>	0.63777936	0.002483336
<i>IQSEC2</i>	<i>AOFI</i>	-0.699029738	0.000605042
<i>IQSEC2</i>	<i>AP1S3</i>	0.710004036	0.000453029
<i>IQSEC2</i>	<i>AP2B1</i>	0.666932075	0.001318852
<i>IQSEC2</i>	<i>APBB1</i>	0.604610546	0.004744394
<i>IQSEC2</i>	<i>APPBP2</i>	-0.641159342	0.002315195
<i>IQSEC2</i>	<i>AQP5</i>	0.649444834	0.001942798
<i>IQSEC2</i>	<i>ARD1B</i>	0.761792746	9.51E-05
<i>IQSEC2</i>	<i>ARF6</i>	0.720673311	0.000337675
<i>IQSEC2</i>	<i>ARFRP1</i>	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	CLASPI	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	DVLI	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	FXYP6	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	HIFX	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	MAFI	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	MESPI	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	PACSI	0.772881919	6.47E-05
IQSEC2	PALM	0.680219205	0.00096616
IQSEC2	PAPD5	0.67934259	0.00098667
IQSEC2	PCLO	0.78432548	4.25E-05

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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

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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	RIFI	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	SBFI	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

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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	SNWI	-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

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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	TCPI1L1	0.748968078	0.000144778
IQSEC2	TEPI	0.611789033	0.004148748
IQSEC2	THAP4	0.620969223	0.00347846
IQSEC2	THAP7	0.641872445	0.002280964
IQSEC2	THGIL	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	TP53111	0.740264418	0.000189984
IQSEC2	TRAF1	-0.629513473	0.002937887
IQSEC2	TRIB2	0.66194054	0.001476704
IQSEC2	TRRAP	0.61984277	0.003555517

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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	TLL1	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

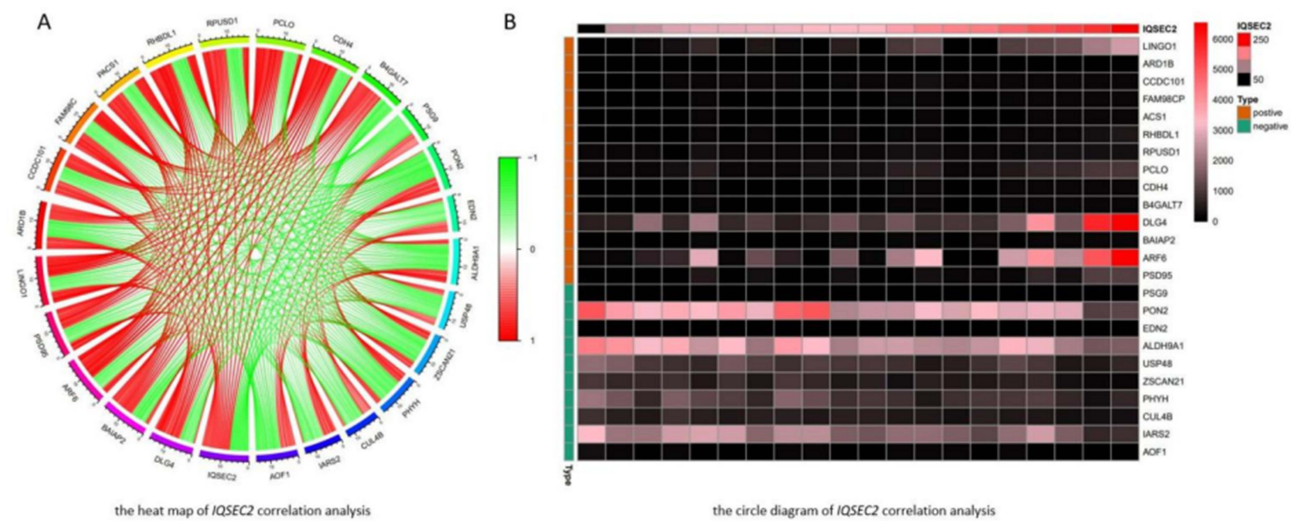


Figure 4 (A) Analysis circle diagram of Top 25 *IQSEC2* related genes, and approaching red indicates positive correlation, approaching green indicates negative correlation. **(B)** The heatmap of *IQSEC2* correlation analysis, and brown represents positive, green represents negative.

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

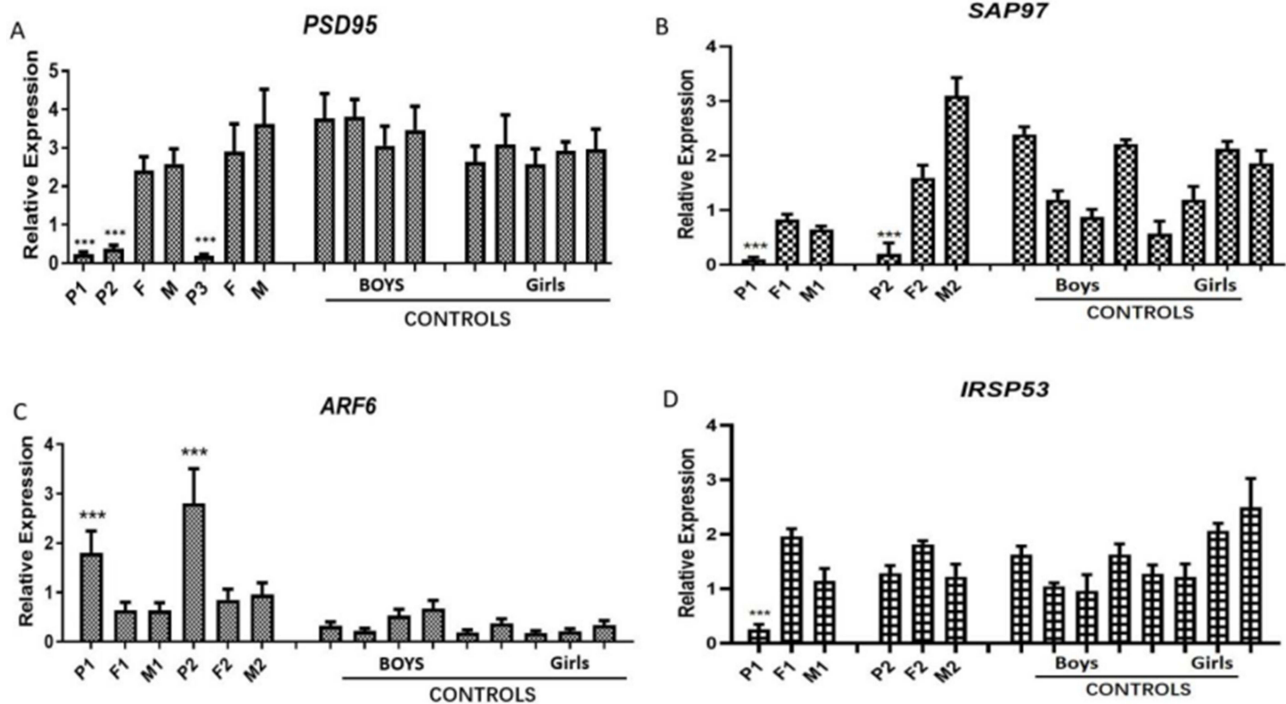


Figure 5 (A) The expression levels of *PSD-95* in probands 1 and 2 were significantly lower than normal levels, which were not observed in their parents. **(B)** The expression levels of *SAP97* in probands 1 and 2 were significantly lower than normal levels, which were not observed in their parents. **(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

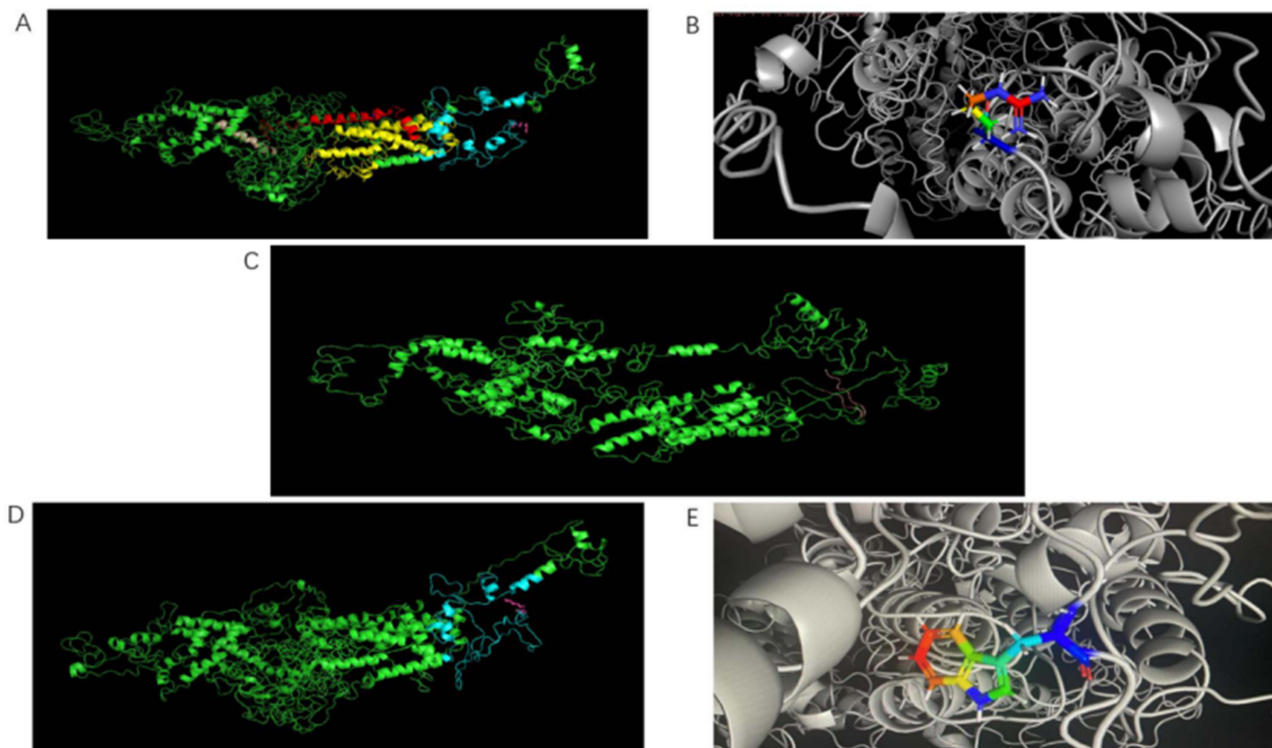


Figure 6 (A) Three-dimensional structure of *IQSEC2* wild-type protein. (B) Fine structure of wild-type amino acid at position 1192. (C) Nonsense mutation c.3576C>A (p. Tyr1192*). (D) Missense mutation c.2983C>T (p. Arg995Trp). (E) Fine structure of missense mutation at position 995.

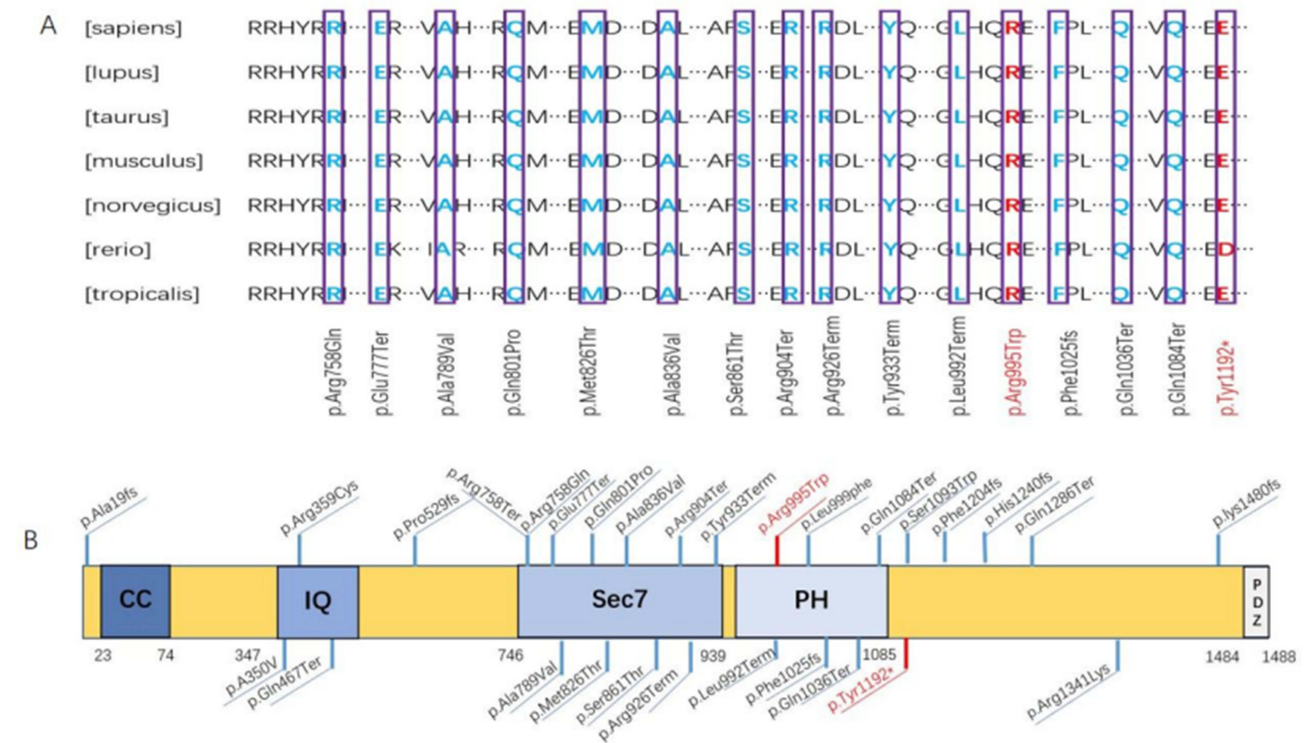


Figure 7 (A) The IQSEC2 protein sequence. Conservative studies were conducted for c.3576C>A (p. Tyr1192*) and c.2983C>T (p. Arg995Trp) in humans, *Canis lupus familiaris*, *Mus musculus*, *Rattus norvegicus*, *Bos Taurus*, *Xenopus tropicalis*, and *Danio rerio*. (B) Primary structure of IQSEC2 and the 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 genome 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 *AMPA* activity-dependent clearance.² However, mutations in the PDZ domain of *IQSEC2* destroy the regulation and neurotransmission of glutamate receptors in hippocampal cultures² 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 transport³ 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 <i>IQSEC2</i>							
PMID	Gene	Variation (cDNA) NM_001111125.2	Variation (Protein) NP_001104595.1	Location	Annotation	Clinical Significance	Related Disorder
26795593;25356970;29100083; 28815955;30206421	<i>IQSEC2</i>	c.804delC	del 1 bp codon 268	NA	deletion	Pathogenic	Epilepsy; Mental retardation, X-linked
24306141;26795593	<i>IQSEC2</i>	c.2052_2053delCG	del 2 bp codon 684	NA	deletion	Pathogenic	Intellectual disability; Epilepsy
27864847;30206421	<i>IQSEC2</i>	c.4039dupG	ins 1 bp codon 1347	NA	duplication	Likely pathogenic	Intellectual disability and epilepsy
27665735;30842726; 29026562	<i>IQSEC2</i>	c.1049C>T	A350V	IQ motif, EF-hand binding site	substitution	Pathogenic	Intellectual disability, nonsyndromic
27652284;30255931; 30206421	<i>IQSEC2</i>	c.2312G>A	G771D	Sec7 domain	substitution	Pathogenic	Intellectual disability and epilepsy
24306141;25356970; 26795593	<i>IQSEC2</i>	c.2582G>C	S861T	Sec7 domain Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability and Epilepsy
28815955;3066632	<i>IQSEC2</i>	c.2507C>T	A836V	Sec7 domain Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability, syndromic
29720203;30206421	<i>IQSEC2</i>	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	<i>IQSEC2</i>	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	<i>IQSEC2</i>	c.2563C>T	R855Term	Sec7 domain Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Intellectual disability, nonsyndromic
27062609;30206421	<i>IQSEC2</i>	c.2776C>T	R926Term	Sec7 domain Sec7 domain, alpha orthogonal bundle	substitution	Pathogenic	Retts-like syndrome;
28815955;30206421	<i>IQSEC2</i>	c.2317C>T	Q773Term	Sec7 domain	substitution	Likely pathogenic	Intellectual disability, syndromic
29100083;30206421	<i>IQSEC2</i>	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. *ARF6* 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 plasticity³⁹ 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.

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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.

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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. Shoubbridge 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. Saján 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, Shekhdem 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, Altiock 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, Geroges 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

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