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Variants in *BSN* gene associated with epilepsy with favourable outcome

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ABSTRACT

Background *BSN* gene encodes Bassoon, an essential protein to assemble the cytomatrix at the active zone of neurotransmitter release. This study aims to explore the relationship between *BSN* variants and epilepsy.

Methods Whole-exome sequencing was performed in a cohort of 313 cases (trios) with epilepsies of unknown causes. Additional cases with *BSN* variants were collected from China Epilepsy Gene V.1.0 Matching Platform. The Clinical Validity Framework of ClinGen was used to evaluate the relationship between *BSN* variants and epilepsy.

Results Four pairs of compound heterozygous variants and one cosegregating heterozygous missense variant in *BSN* were identified in five unrelated families. These variants presented statistically higher frequency in the case cohort than in controls. Additional two de novo heterozygous nonsense variants and one cosegregating heterozygous missense variant were identified in three unrelated cases from the gene matching platform, which were not present in the Genome Aggregation Database. The missense variants tended to be located in C-terminus, including the two monoallelic missense variants. Protein modelling showed that at least one missense variant in each pair of compound heterozygous variants had hydrogen bond alterations. Clinically, two cases were diagnosed as idiopathic generalised epilepsy, two as focal epilepsy and the remaining four as epilepsy with febrile seizures plus. Seven out of eight probands showed infancy or childhood-onset epilepsy. Eight out of 10 affected individuals had a history of febrile convulsions. All the cases were seizure-free. The cases with monoallelic variants achieved seizure-free without treatment or under monotherapy, while cases with biallelic missense variants mostly required combined therapy. The evidence from ClinGen Framework suggested an association between *BSN* variants and epilepsy.

Conclusion The *BSN* gene was potentially a novel candidate gene for epilepsy. The phenotypical severity was associated with the genotypes and the molecular subregional effects of the variants.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Bassoon protein encoded by *BSN* gene plays a vital role in releasing synaptic vesicles. The relationship between *BSN* variants and human diseases remains unknown.

WHAT THIS STUDY ADDS

⇒ Four pairs of compound heterozygous variants, two de novo heterozygous nonsense variants and two heterozygous missense variants with cosegregation were identified in eight patients with epilepsies with favourable outcome. The pathogenic missense variants tended to gather at the C-terminus of Bassoon.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The *BSN* gene is potentially a novel candidate gene for epilepsy. Its phenotypical severity was associated with the genotypes and the molecular subregional effects of missense variants.

INTRODUCTION

Epilepsy is a common neurological disorder featured by the recurrence of unprovoked seizures. The aetiology of epilepsy is classified into genetic, structural, infectious, immunological, metabolic and unknown aetiologies.[1] Genetic causes account for 70%–80% of epilepsy cases,[2] with more than 977 genes being related to epilepsy.[3] Recently, dozens of new causative genes for epilepsy have been identified, such as *PGM3*,[4] *CHD4*,[5] *UNC13B*,[6] *CELSR3*,[7] *RHOBTB2*[8] and *SYNGAP1*.[9] However, the causative genes in the majority of patients still remain unknown.[10]

The *BSN* gene (OMIM* 604020), located on 3q21.31, encodes the Bassoon protein, which is highly expressed in the mammalian brain, especially in the cerebral cortex and the hippocampus. Bassoon is involved in synaptic vesicles trafficking and establishing the cytomatrix at the active zone (CAZ).[11–14] It plays a vital role in maintaining synapse integrity,[15] participating in the formation of Piccolo-Bassoon transport vesicles (PTVs)[16] and regulating the activity at both inhibitory and excitatory glutamatergic synapses in the cortex and hippocampus.[17] Mice of homozygous *BSN* null led to spontaneous seizures and partial premature lethality.[17 18] However, the relationship between *BSN* variants and human diseases remains unknown.

In this study, trio-based whole-exome sequencing (WES) was performed in a cohort of 313 cases with epilepsies of unknown causes. Novel *BSN* variants were identified in five cases. These variants presented at a statistically higher frequency in the case cohort than in controls. Three additional variants without allelic frequency in Genome Aggregation Database (gnomAD) were identified in

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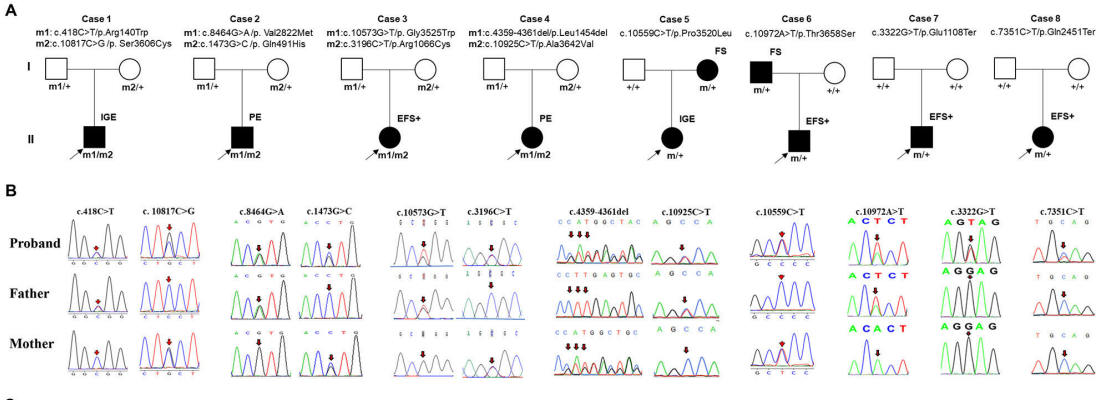

Figure 1 Genetic data of cases with *BSN* variants. (A) Pedigrees of the eight cases with *BSN* variants and their corresponding phenotypes. (B) DNA sequence chromatogram of the *BSN* variants. Arrows indicate the positions of the variants. EFS+, epilepsy with febrile seizures; FS: febrile seizure; IGE, idiopathic generalised epilepsy; PE, partial epilepsy.

three cases coming from different epilepsy centres. This study suggested that *BSN* was potentially a candidate gene of epilepsy.

MATERIALS AND METHODS

Subjects

A total of 313 cases of epilepsy with unknown causes were recruited from the Epilepsy Centre of the Second Affiliated Hospital of Guangzhou Medical University in China from 2018 to 2020. Patients with acquired aetiologies, such as tumour, trauma, infection, immunity and stroke, were excluded. Clinical information of the affected individuals was collected, including age of onset, seizure type and frequency, family history, general and neurological examination results, and response to antiepileptic drugs. Brain MRI scans were performed to detect structural abnormalities. Long-term video EEG monitoring records that included open–close eyes test, hyperventilation, intermittent photic stimulation and sleeping recording were obtained according to the EEG technical guideline.[19] The EEG results

Table 1 Analysis of the aggregate frequency of *BSN* variants identified in this study

Identified *BSN* variants		Allele count/number in this study	Allele count/number in gnomAD-all populations	Allele count/number in gnomAD-East Asian populations	Allele count/number in controls of gnomAD-all populations	Allele count/number in controls of gnomAD-East Asian populations	Homozygote count/number in gnomAD-all populations
Variants from Epilepsy Centre of the Second Affiliated Hospital of Guangzhou Medical University							
Case 1	Chr3:49662601:c.418C>T/p.Arg140Trp	1/626 (0.0016)	10/221 818 (0.00004508)	4/16 050 (0.0002492)	5/97 838 (0.00005110)	2/8344 (0.0002397)	0
	Chr3:49700408:c.10817C>G/p.Ser3606Cys	1/626 (0.0016)	1/251 264 (0.000003980)	1/18 388 (0.00005438)	–/–	–/–	0
Case 2	Chr3:49680540:c.1473G>C/p.Gln491His	1/626 (0.0016)	–/–	–/–	–/–	–/–	–/–
	Chr3:49695453:c.8464G>A/p.Val2822Met	1/626 (0.0016)	3/251 058 (0.00001195)	0/18 392 (0)	2/109 322 (0.00001829)	0/9046 (0)	0
Case 3	Chr3:49690185:c.3196C>T/p.Arg1066Cys	1/626 (0.0016)	23/230 922 (0.00009960)	1/18 510 (0.00009960)	10/87 760 (0.0001139)	0/8954 (0)	0
	Chr3:49699851:c.10573G>T/p.Gly3525Trp	1/626 (0.0016)	–/–	–/–	–/–	–/–	–/–
Case 4	Chr3:49691347:c.4359_4361del/p.Leu1454del	1/626 (0.0016)	2/251 342 (0.000007957)	2/18 394 (0.0001087)	1/109 382 (0.000009142)	1/9046 (0.0001105)	0
	Chr3:49700516:c.10925C>T/p.Ala3642Val	1/626 (0.0016)	1/248 246 (0.000004028)	1/18 278 (0.00005471)	1/108 034 (0.000009256)	1/8974 (0.0001114)	0
Case 5	Chr3:49700563:c.10559C>T/p.Pro3520Leu	1/626 (0.0016)	–/–	–/–	–/–	–/–	–/–
Total		9/626 (0.0144)	40/221 818 (0.00018)	9/16 050 (0.00056)	19/87 760 (0.00022)	4/8344 (0.00048)	0
P value			$1.943e^{-14}$	$5.02e^{-09}$	$2.595e^{-13}$	$2.06e^{-08}$	
OR (95% CI)			80.79797 (34.35069–169.99028)	25.97851 (9.102645–74.285905)	67.30892 (26.73007–156.66261)	30.38158 (8.451071–135.366707)	
Variants from China Epilepsy Gene V.1.0 Matching Platform							
Case 6	Chr3:49700563:c.10972A>T/p.Thr3658Ser		–/–	–/–	–/–	–/–	–/–
Case 7	Chr3:49690311:c.3322G>T/p.Glu1108Ter		–/–	–/–	–/–	–/–	–/–
Case 8	Chr3:49694340:c.7351C>T/p.Gln2451Ter		–/–	–/–	–/–	–/–	–/–

P values and ORs were estimated with two-sided Fisher's exact test.
gnomAD, Genome Aggregation Database.

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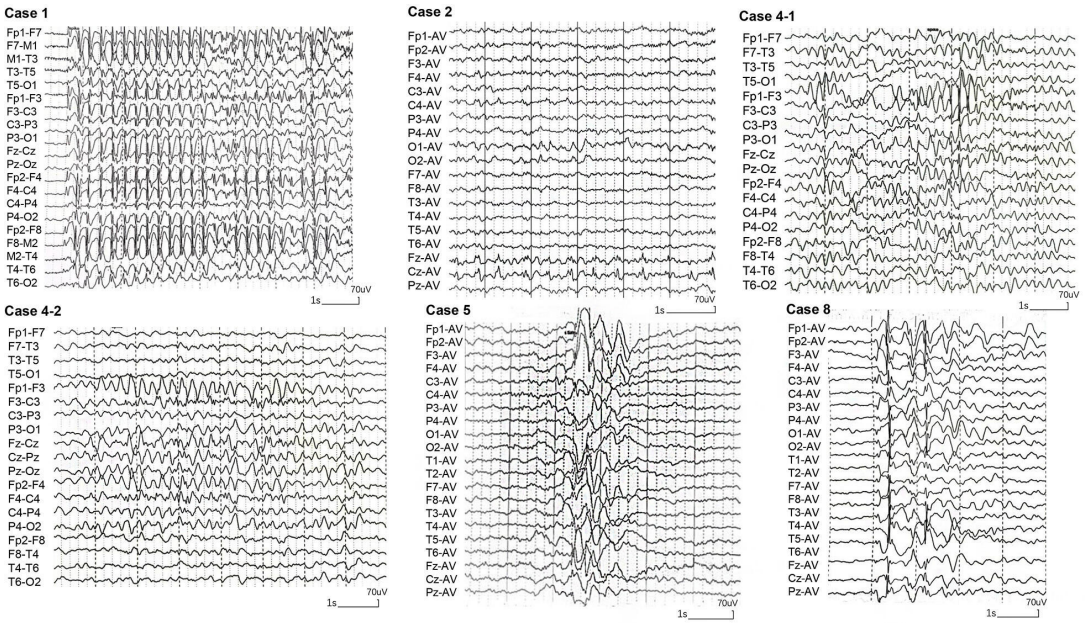

Figure 2 Representative interictal EEG in the cases with *BSN* mutations. Interictal EEG of case 1 showed generalised 3–4 Hz spike slow waves. Interictal EEG of case 2 showed central midline area discharges. Interictal EEG of case 4 showed left frontal discharges and bilateral frontocentral slow activities. Interictal EEG of case 5 showed irregular generalised spike-and-slow waves. Interictal EEG of case 8 showed generalised spike-and-slow waves.

were reviewed by two qualified electroencephalographers. Epileptic seizures and epilepsy syndromes were diagnosed according to the criteria of the Commission on Classification and Terminology of the International League Against Epilepsy (1981, 1989, 2001, 2010, 2017 and 2022). All of the subjects were followed up to at least 1 year.

WES and bioinformatic analyses

Blood samples were obtained from the probands and their parents and other family members, if available. The genomic DNAs were extracted by using Qiagen Flexi Gene DNA kit (Qiagen, Hilden, Germany). WES was performed with NextSeq2000 sequencing instruments (Illumina, San Diego, California, USA). Sequence alignment and variant calling were performed according to standard procedures as previously described.[4 5 20] The potentially disease-causing variants were analysed with an individualised protocol. Included variants in this study should meet the following criteria: (1) minor allele frequencies of <0.005 according to gnomAD in the general population or East Asian population; (2) the total minor allele frequency of biallelic variants of compound heterozygotes is $<10^{-7}$. The variants in each trio were sorted according to the following five models: (1) epilepsy-associated gene model; (2) de novo variant dominant model; (3) autosomal recessive inheritance model, including homozygous and compound heterozygous variants; (4) X-linked model; and (5) cosegregation analysis model. To identify novel epilepsy-associated genes, the known epilepsy-associated genes were put aside.[3] Genes with de novo variants, biallelic variants, hemizygous variants or variants with segregations, which represented the genetic difference between the patients and normal individuals in a family and potentially explained the occurrence of disease, were selected for further studies to define the gene–disease association. The *BSN* gene emerged as one of the candidate genes with recurrent compound heterozygous variants and variants with segregations in this cohort. All candidate pathogenic variants were validated by Sanger sequencing. The variants in the *BSN* gene were annotated based on the transcript NM_003458.3. Multiple web-based in silico programs were used to evaluate the damaging effect of candidate variants in the *BSN* gene.

Validation of *BSN* variants in epilepsy

To verify the correlation between *BSN* variants and epilepsy, additional cases with epilepsy and *BSN* variants were collected from China Epilepsy Gene V.1.0 Matching Platform. All cases with epilepsy had neither acquired causes nor pathogenic variants in known epilepsy-associated genes.[3]

Protein modelling and stability

To evaluate the pathogenicity of candidate variants, in silico modelling of Bassoon was conducted by phyre2 Protein Fold Recognition Server (http://www.sbg.bio.ic.ac.uk/phyre2/). As Bassoon is a large protein with 3926 amino acids, the target protein was divided into three parts for analysis. PyMOL (https://www.pymol.org) was used for visualising the crystal structure and introducing amino acid changes caused by the variants. The I-Mutant V.3.0 (http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) was used to predict the effect of the variants on the stability of the protein structure.

Statistical analysis

The data were processed by R statistical software V.3.4.1. The comparison in frequency of variants between the case cohort and controls was conducted by Fisher's exact method. The burden of recessive variants was analysed according to the method recommended by Martin *et al*.[21] A p value of <0.05 was regarded as statistical significance.

Evaluating the *BSN* gene as a novel candidate epilepsy gene

The Clinical Validity Framework developed by Clinical Genome Resource (ClinGen) was used to evaluate *BSN* as a novel candidate epilepsy gene from both genetic and experimental aspects.[22]

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Table 2 Clinical features of the individuals with *BSN* variants

Case	Variants (NM_003458.3)	Gender	Seizure onset	Seizure course	Seizure outcome	Effective AEDs	EEG	Brain imaging	Development	Diagnosis
Case 1	c.418C>T/p.Arg140Trp c.10817C>G/p.Ser3606Cys	Male	Early adolescence	GTCS once, absence 1–2 times/day, myoclonus one time/2–3 months	Sz free 1 year	VPA, LEV	Generalised spike-and-wave discharges	Normal	Normal	IGE
Case 2	c.1473G>C/p.Gln491His c.8464G>A/p.Val2822Met	Male	Early childhood	FS once, CPS 1–2 times/night	Sz free 2 years	CBZ, CZP, VPA	Right centroparietal area discharges	Normal	Normal	PE
Case 3	c.3196C>T/p.Arg1066Cys c.10573G>T/p.Gly3525Trp	Female	Toddler	FS 5–6 times/year, then afebrile sGTCS twice/year since 5 years old	Sz free 2 years	LEV	Normal	Normal	Normal	EFS+
Case 4	c.4359-4361del/p.Leu1454del c.10925C>T/p.Ala3642Val	Male	Toddler	sGTCS six times/week, CPS 11 times/month	Sz free 7 mos	LEV, LTG, VPA	Left frontal discharges and bilateral frontal–parietal slow activities	Normal	Developmental delay and microcephalus	PE
Case 5–1	c.10559C>T/p.Pro3520Leu	Female	Infancy	FS at 1 year, afebrile GTCS 4 times/year since 14 years old	Sz free 9 years	TPM	Irregular generalised spike-and-slow waves	Normal	Normal	IGE
Case 5–2 (M)	c.10559C>T/p.Pro3520Leu	Female	Infancy	FS 3 times	Sz free	Untreated	NA	NA	Normal	FS
Case 6–1	c.10972A>T/p.Thr3658Ser	Male	Infancy	FS 3–4 times/year, afebrile GTCS twice within 2 years	Sz free 1 year	LEV	Normal	Normal	Normal	EFS+
Case 6–2 (F)	c.10972A>T/p.Thr3658Ser	Male	Infancy	FS once	Sz free	Untreated	NA	NA	Normal	FS
Case 7	c.3322G>T/p.Glu1108Ter	Male	Toddler	FS 5–6 times/year	Sz free 3 year	Untreated	Normal	Normal	Normal	EFS+
Case 8	c.7351C>T/p.Gln2451Ter	Female	Infancy	FS 3–4 times/year, CPS 4 times/months since 3 years old	Sz free 9 months	LEV	Generalised spike-and-slow waves	Normal	Normal	EFS+

AED, antiepileptic drug; CBZ, carbamazepine; CPS, complex partial seizure; CZP, clonazepam; EFS+, epilepsy with febrile seizures plus; F, father; FS, febrile seizure; GTCS, generalised tonic–clonic seizure; IGE, idiopathic generalised epilepsy; LEV, levetiracetam; LTG, lamotrigine; M, mother; NA, not available; PE, partial epilepsy; sGTCS, secondary generalised tonic–clonic seizure; Sz, seizure; TPM, topiramate; VPA, valproate.

RESULTS

Identification and analysis of *BSN* variants

Nine novel variants in *BSN* were identified in five unrelated cases (cases 1–5) from the Epilepsy Centre of the Second Affiliated Hospital of Guangzhou Medical University, including four pairs of compound heterozygous variants (c.418C>T/p.Arg140Trp, c.10817C>G/p.Ser3606Cys; c.1473G>C/p.Gln491His, c.8464G>A/p.Val2822Met; c.3196C>T/p.Arg1066Cys, c.10573G>T/p.Gly3525Trp; and c.4359-4361del/p.Leu1454del, c.10925C>T/p.Ala3642Val) and one heterozygous missense variant with cosegregation (c.10559C>T/p.Pro3520Leu) (figure 1A,B). The nine variants were present at low or no allele frequencies in all populations or East Asian populations. There was statistical difference between the frequencies in the cohort of epilepsy and that in the populations in gnomAD (9/626 vs 40/221 818 in all populations of gnomAD, $p=1.943\times10^{-14}$; vs 9/16 050 in the East Asian population of gnomAD, $p=5.02\times10^{-9}$; vs 19/87 760 in controls of gnomAD-all populations, $p=2.595\times10^{-13}$; vs 4/8344 in controls of gnomAD-East Asian populations of gnomAD, $p=2.06\times10^{-8}$). None of them was discovered in the homozygotes of all population according to gnomAD (table 1). When the burden of recessive variants was analysed, the *BSN* variants in the present cohort were significantly more than the expected number by chance in the East Asian population (minor allele frequency from gnomAD (MAF) <0.005, $p=2.316\times10^{5}$).

Three additional novel *BSN* variants were identified in three unrelated cases (cases 6–8) from the China Epilepsy Gene V.1.0 Matching Platform, including two de novo nonsense variants (c.3322G>T/p.Glu1108Ter and c.7351C>T/p.Gln2451Ter) and one missense heterozygous variant with cosegregation (c.10972A>T/p.Thr3658Ser) (figure 1A,B). The three variants have not been recorded in gnomAD.

All the probands did not carry any other pathogenic or likely pathogenic variant in the genes known to be associated with epilepsy.[3]

Clinical features of the cases with *BSN* variants

In total, *BSN* variants were identified in eight cases involving 10 affected individuals. All probands showed infancy or childhood-onset epilepsy (the onset age ranging from 10 months old to 3 years old), with the exception in case 1 who started seizures at early adolescence. Eight out of 10 affected

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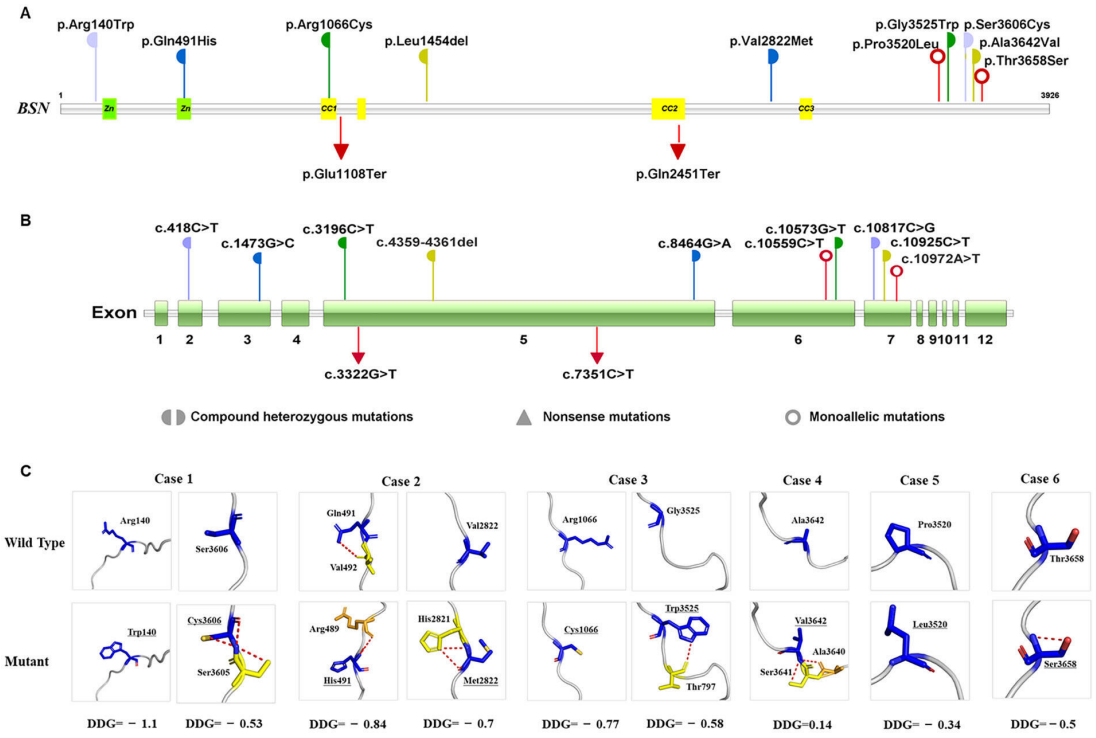

Figure 3 Schematic illustration of variants, interactions with surrounding amino acids and protein stability prediction. (A) Schematic diagram of Bassoon structure and the localisation of the *BSN* variants identified in this study. (B) Schematic diagram of the *BSN* gene. The two nonsense variants (p.Glu1108Ter and p.Gln2451Ter) occurred in exon 5, which would result in the truncation of Bassoon protein and trigger nonsense-mediated decay. (C) Hydrogen bond changes and DDG value of *BSN* variants. DDG <0: decreased protein stability; DDG >0: increased protein stability. Six of the nine missense variants led to hydrogen bond alterations. Residue Ser3606 had no hydrogen bond, but the mutant residue Cys3606 formed three hydrogen bonds (one with Ser3605 and two interconnect internally). Residue Gln491 was initially connected with Val492 by a hydrogen bond, while hydrogen bond was altered to connect with residue Arg489 in the mutant residue. Residue Met2822 originally had no hydrogen bond, but the mutant residue Met2822 formed two hydrogen bonds with residue His2821. When threonine was replaced by serine at 3658, one hydrogen bond had formed immanently. Residue Arg140, Arg1066 and Pro3520 formed no hydrogen bond with the surrounding residues in both wild type and mutant residues.

individuals had a history of febrile convulsions. All the cases but case 4 had normal development. The brain MRI in all the cases was normal. The representative EEG is shown in figure 2. Their clinical features are summarised in table 2.

Two probands (cases 1 and 5) were diagnosed as idiopathic generalised epilepsy, two (cases 2 and 4) as partial/focal epilepsy and the remaining four probands (cases 3 and 6–8) as epilepsy with febrile seizures plus. Additionally, the parents of cases 5 and 6 carrying *BSN* variants had febrile seizures.

All the cases were seizure-free. Among the four cases with monoallelic variants (cases 5–8), case 7 with p.Glu1108Ter was seizure-free without treatment, while the other three cases (cases 5, 6 and 8) were seizure-free with monotherapy. Among the four cases with biallelic variants (cases 1–4), one case (case 3) achieved seizure-free status with monotherapy, while three cases (cases 1, 2 and 4) were seizure-free with combined therapy. Case 4 with the deletion variant (p.Leu1454del) and the missense variant in C-terminus (p.Ala3642Val) presented frequent seizures and developmental delay (he could not sit alone and speak at early childhood). He was microcephalic with an occipitofrontal head circumference of 44.5 cm (−3.3 SD) at early childhood. He was finally seizure-free under the combination of levetiracetam, lamotrigine and valproate.

Consequence of *BSN* variants and the genotype–phenotype correlation

Bassoon is constituted of 3926 amino acids, including two zinc finger motifs in the N-terminal region, three coiled-coil domains in the central part and a stretch of polyglutamines at its C-terminus.[23]

The two nonsense variants (c.3322G>T/p.Glu1108Ter and c.7351C>T/p.Gln2451Ter) were located close to the first and second coiled-coil domains, respectively (figure 3). They both occurred in exon 5 (of the 12 exons), were predicted to result in truncation of Bassoon protein and trigger nonsense-mediated decay.[24] They were de novo and produced clinical symptoms by monoallelic variants. The probability of being loss-of-function intolerant (pLI) of *BSN* gene is 1.0; the loss-of-function observed/expected upper bound fraction (LOEUF) is 0.11 (0.07–0.17); and the probability of haploinsufficiency (pHI) is 0.819,[25] indicating loss of function and haploinsufficiency of *BSN* are potentially pathogenic.

As shown in figure 3A, four missense variants (p.Arg140Trp, p.Gln491His, p.Arg1066Cys and p.Val2822Met) scattered in different domains of Bassoon protein, while five missense variants (p.Pro3520Leu, p.Gly3525Trp, p.Ser3606Cys, p.Ala3642Val and p.Thr3658Ser), including the two monoallelic variants, were clustered in the C-terminus, potentially suggesting the functional particularity of C-terminus.

The analysis of crystal structures of Bassoon protein by PyMol showed that six out of the nine missense variants led to hydrogen bond alterations with surrounding residues (figure 3B). Among the four pairs of compound heterozygous variants, at least one variant in each pair had hydrogen bond alterations. Case 4 had the compound heterozygous variant

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Table 3 Evaluating the clinical validity of *BSN*–epilepsy associations based on the framework developed by the Clinical Genome Resource

	Evidence type	Case information type (suggested starting score)		Suggested upgrades		Scoring range	Points given	Max score
				Functional data		De novo		
Case-level data	Variant evidence	Predicted or proven null variant (1.5 points)		+0.5 points	+0.5 points	0–3 points (per variant)	**5.5***	**12 points**
		Other variant type (0.1 points)		+0.4 points	+0.4 points	0–1.5 points (per variant)	**0.9†**	
	Segregation evidence	Evidence of segregation in one or more families		Sequencing Method		0–3 points	–	**3 points**
			Total LOD Score	Candidate gene Sequencing	Exome/Genome or all genes sequenced in linkage region			
			2–2.99	0.5 points	1 points			
			3–4.99	1 points	2 points			
			>5	1.5 points	3 points			
Case–control data	**Case–control study type**	**Case–control quality criteria**		**Suggested points/study**			**Points given**	**Max score**
	Single variant analysis	► Variant detection methodology. ► Power. ► Bias and confounding factors. ► Statistical significance.		0–6 points			–	12 points
	Aggregate variant analysis			0–6 points			–	
Total allowable points for genetic evidence							6.4	12 points

Evidence category	Evidence type	Suggested points		Points given	Max score
		Default	Range		
Function	Biochemical function	0.5	0–2	0.5‡	2
	Protein interaction	0.5	0–2	0.5§	
	Expression	0.5	0–2	0.5¶	
Functional alteration	Patient cells	1	0–2	0	2
	Non-patient cells	0.5	0–1	0.5**	
Models	Non-human model organism	2	0–4	4††	4
	Cell culture model system	1	0–2	0	
Rescue	Rescue in human	2	0–4	0	
	Rescue in non-human model organism	2	0–4	0	
	Rescue in cell culture model	1	0–2	0	
	Rescue in patient cells	1	0–2	0	
Total allowable points for experimental evidence				6	6
Clinical validity summary matrix				12.4	Strong

*Two de novo null variants and one inherited small deleting variant (2 points/variant×2 variants+1.5 points/variant×1 variant)
†Nine inherited missense variants (0.1 point/variant×9 variants).
‡Neurotransmitter is vital for the neuron excitability. BSN protein participates in the formation of Golgi-derived membranous organelles termed Piccolo-Bassoon transport vesicles that are transported along axons to sites of nascent synaptic contacts. It is crucial for the development and survival of a wide range of neuronal cells (assigned 0.5 point).
§BSN protein is one of the members assembled at the presynaptic cytomatrix at the active zone, in which BSN protein combines with Munc13 proteins. Loss of Munc13-1 function causes cortical hyperexcitability. The mutations in UNC13B gene which encode Munc13-2 protein were associated with epilepsy (assigned 0.5 points).
¶BSN gene is highly expressed in the mammalian brain especially in the cerebral cortex (assigned 0.5 point).
**Experimentally, a genetic deletion of Bassoon or an acute interference with Bassoon-RBP interaction reduces synaptic abundance of CaV2.1, weakens P/Q-type Ca^{2+} current-driven synaptic transmission, and results in higher relative contribution of neurotransmission dependent on CaV2.2. Another experiments show that the absence of Bassoon led to the inactivation of a significant fraction of glutamatergic synapses, induced a reduction in normal synaptic transmission, and resulted in that vesicles were clustered and docked in normal numbers but were unable to fuse (assigned 0.5 point).
††Mice of homozygous BSN null led to partial premature lethality and spontaneously seizures (MGI: 1277955, assigned 4 points).
LOD, log of odds (genetic linkage score).

constituted by an in-frame deletion variant (p.Leu1454del) and a missense variant (p.Ala3642Val) located in the C-terminus with hydrogen bond alterations. The patient presented frequent seizures, neurodevelopmental delay and microcephalus.

Evaluation of epilepsy as a novel phenotype of *BSN* variants

To evaluate the correlation between *BSN* gene and epilepsy, the assessment was performed based on the framework developed by the National Institutes of Health-funded ClinGen.[22] There were two different aspects of this framework involved: genetic and experimental, having scores of 6.4 and 6.0, respectively. Gene–disease relationship scores for these two aspects were 12.4, which was ranked as 'strong', suggesting that the *BSN* gene was closely associated with epilepsy (table 3).

DISCUSSION

The *BSN* gene, also referred to as *ZNF231*, is the presence of 12 coding exons spreading over 350 kb of genomic DNA. *BSN* gene encodes Bassoon, which is highly expressed in the cerebral cortex and the hippocampus during infant stage. Bassoon is one of assembling proteins to form the presynaptic cytoskeleton at vesicular release sites—CAZ. CAZ is a meshwork of cytoskeleton

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underlying the plasma membrane and is composed by multiple proteins, including Bassoon, Piccolo, Munc13 proteins, RIMs and CAST.[26] Previous studies indicated that loss of function of Munc13-1 caused cortical hyperexcitability by leaving syntaxin in a non-functional closed state.[27] Our previous study showed that deficiency of the Munc13-2 protein was associated with partial epilepsy.[6] These findings suggested that CAZ proteins were associated with epilepsy. Experimentally, Bassoon was required for normal recruitment of P/Q-type of voltage-gated calcium channel and regulation of neurotransmitter release.[13] In animal models, *BSN*$^{-/-}$ mutant mice developed pronounced seizures.[18] Recently, a genome-wide association study implicated one *BSN* intronic variant (rs1191743) with febrile seizures,[28] indicating the *BSN* variants of low MAF were potentially associated with seizure susceptibility. In this study, *BSN* variants were identified in eight cases with epilepsy. Further assessment of the ClinGen Clinical Validity Framework confirmed an association between *BSN* variants and epilepsy.

The pLI, LOEUF and pHI of the *BSN* gene indicated loss of function and haploinsufficiency were potentially pathogenic.[22 29] Experimentally, the absence of Bassoon led to the inactivation of a significant fraction of glutamatergic synapse which induced a reduction in normal synaptic transmission and resulted in vesicles being clustered and docked in normal numbers but unable to fuse.[18] In *BSN*$^{-/-}$ mutant mice, interictal spikes were recorded in the cortex and hippocampus. Phenotypically, *BSN*$^{-/-}$ mutant mice exhibited spontaneous epileptic seizures, even died from seizures, suggesting a pathogenic role of *BSN* loss of function. In the present study, both truncating variants and compound heterozygous variants were identified in the patients with epilepsy. The truncating variants resulted in a loss of function by yielding a premature termination of the protein. Among the compound heterozygous variants, at least one variant in each pair of biallelic variants led to the alterations of hydrogen bonds with surrounding amino acids or the changes of protein length, implying functional damages of Bassoon. Thus, loss of function of Bassoon was potentially the underlying mechanism of the pathogenicity of *BSN* variants, which is consistent with the pathogenesis of other genes encoding CAZ proteins, such as *UNC13A*[27] and *UNC13B*.[6] However, the functional effects of the *BSN* variants warrant further investigation.

In animals, the heterozygous *BSN* knockout mice model showed no phenotype, while the homozygous knockout mice showed abnormal central nervous system synaptic transmission and seizures, even preweaning lethality. Clinically, the patients with monoallelic *BSN* missense variants achieved seizure-free status without treatment or under monotherapy, while the majority of patients with biallelic missense variants required combined therapy, and case 4 with the deletion variant (p.Leu1454del) and the missense variant (p.Ala3642Val) located in the C-terminus with hydrogen bond alterations even presented developmental abnormalities, indicating a correlation between genotype and phenotype severity. Considering homozygous *BSN* knockout mice displaying preweaning lethality, it is possible that biallelic null variants in human were fatal. Therefore, the epileptic phenotype spectrum of *BSN* variants potentially ranged from benign epilepsy caused by monoallelic missense variants in the mild extreme to severe epilepsy with neurodevelopmental delay (even fatal epilepsy) associated with biallelic variants of severe damage in the other extreme.

The pathogenic missense variants tended to gather at the C-terminus of Bassoon; especially the two monoallelic missense variants (p.Pro3520Leu and p.Thr3658Ser) were also located in the C-terminus, indicating a subregional effect. Physiologically, Bassoon deposits at synapses, fuses with the presynaptic plasma membrane by exocytotic of the transport vesicle, then directly places the C-terminus close to CAZ. In the process, the C-terminus of Bassoon is attached to equivalent membranes (Golgi membranes and subsequently to the PTV membrane) all along the trafficking route.[11 30] The damages of the C-terminus may directly affect docking, fusing and trafficking of synaptic vesicles, explaining the potentially severe pathogenicity of the missense variants in the C-terminus.

Among the 4657 disease-causing genes in humans (OMIM, www.omim.org), 539 genes (11.6%) were associated with genetic disorders in both dominant and recessive inheritance patterns. The coexistence of dominant and recessive inheritance mode is common in the epilepsy-related genes, such as *SLC12A5*, *RELN*, *EFHC1*, *CPA6*, *KCNMA1* and *SCN1B*. In the present study, the truncating variants were of de novo and the two missense variants located in the C-terminus were cosegregating in the families, while the other missense variants were inherited in recessive inheritance pattern, indicating the correlation between *BSN* genotypes and inheritance.

It is worth noting that all of the cases were finally seizure-free, although case 4 presented frequent seizures initially. Seizures in the majority of cases started before 3 years old and remitted at the adolescence stages. *BSN* was significantly highly expressed in infant stage and reduced in adolescence and adult.[31] The consistence between temporal pattern of gene expression and occurrence of seizures potentially implies clinical significance in evaluation of the course and outcome of epilepsy.

In conclusion, this study suggested that the *BSN* gene was potentially a candidate gene of epilepsy with favourable outcome. The phenotypical severity of *BSN* variants was associated with their genotypes, such as missense or destructive mutations, monoallelic or biallelic mutations, and the molecular subregional effects of missense variants.

Correction notice This article has been corrected since it was published Online first. In the 'Subjects' section, 'from 2012 to 2020' has been corrected to 'from 2018 to 2020'.

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