A De Novo SEMA6B Variant in a Chinese Patient with Progressive Myoclonic Epilepsy-11 and Review of the Literature

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Abstract
Progressive myoclonic epilepsy is a group of neurodegenerative diseases with complex clinical and genetic heterogeneity, which is associated with spontaneous or action-induced myoclonus and progressive neurodegeneration. Since 2020, 4 families with progressive myoclonic epilepsy-11 [OMIM#618876] have been reported with a very limited spectrum of SEMA6B pathogenic variants. In our study, whole-exome sequencing was used in a proband from a non-consanguineous Chinese family presenting with growth retardation and recurrent atonic seizures. A deletion mutation (c.1960_1978del, p.Leu654Argfs*25) in the last exon of SEMA6B was detected, which is a de novo variant and pathogenic. The new genetic evidence we reported here strengthened the gene-disease relationship, and the gene curation level between SEMA6B and progressive myoclonic epilepsy-11 became “strong” following the ClinGen SOP. Therefore, the results of this study broaden the mutation spectrum of SEMA6B in different ethnic groups and strengthen the gene-disease relationship between SEMA6B and progressive myoclonic epilepsy-11.

Keywords SEMA6B · Progressive myoclonic epilepsy · Exome sequencing · Gene curation

Introduction
Progressive myoclonic epilepsy (EPM) is a group of neurodegenerative diseases with complex clinical and genetic heterogeneity that is associated with spontaneous or action-induced myoclonus and progressive neurodegeneration (Dijk and Tijssen 2010; Satishchandra and Sinha 2010; Franceschetti et al. 2014; Kälviäinen 2015; Malek et al. 2015). Neurological deterioration may include neuropathy, ataxia, progressive cognitive regression, and myopathy. Although myoclonic seizures are a core feature, other types of seizures occur frequently, including generalized tonic–clonic, tonic, and atypical absence seizures. Other types of epilepsy are often more problematic than myoclonic seizures (Holmes 2020).

Most of the diseases are inherited in an autosomal-recessive manner, and a few show autosomal-dominant inheritance or mitochondrial inheritance. In some patients with EPM, the etiology cannot be determined. However, in recent years, with the development of molecular genetics techniques, especially the clinical application of next-generation sequencing technology, it has been found that mutations in many genes (such as GOSR2, ASAH1, KCTD7, TBC1D24, SCARB2, PRICKLE1, CARS2, SERPINI) can lead to EPM. Very limited case reports have shown that pathogenic variants in semaphorin 6B (SEMA6B)
can also cause progressive myoclonic epilepsy-11 [EPM-11; OMIM#618876]. Hamanaka et al. found three de novo heterozygous frameshift mutations in the last exon of the SEMA6B gene that occurred in four patients from four independent families (Hamanaka et al. 2020), and none in Chinese patients so far. The narrow mutation spectrum of SEMA6B in patients with progressive myoclonic epilepsy has limited our understanding of the relationship between SEMA6B and this disease.

Here, we report a Chinese girl with a de novo mutation in SEMA6B who presented with growth retardation and recurrent atonic seizures, as well as abnormal electroencephalogram (EEG) waves; thus, the gene (SEMA6B)-disease (EPM) relationship was “moderate” according to the ClinGen gene curation protocol. The reporting of this case elevates the level of gene-disease association from “moderate” to “strong,” thus further strengthening the causal relationship between SEMA6B and EPM-11.

Materials and Methods

Participants

A nonconsanguineous Chinese family including a EPM-11 sufferer was recruited at Children's Hospital Affiliated to Soochow University, Suzhou, Jiangsu, China. The proband was diagnosed by whole-exome sequencing. Peripheral blood samples were collected from EPM-11 patient and her parents. Clinical data included available medical histories, neuro-imaging, electroencephalogram, and gene detection. The study followed the principles of the Declaration of Helsinki and was approved by the Institutional Review Committee of Children's Hospital Affiliated to Soochow University. Each participant provided written informed consent.

Whole-Exome Sequencing

Genomic DNA was extracted from peripheral blood samples. xGen Exome Research Panel v1.0 (IDT) was used to capture the target, according to the manufacturer’s instructions, and an Illumina NovaSeq6000 (Illumina) sequenced the DNA. After the analysis and screening of the genetic disease precision diagnosis cloud platform system integrating molecular biology annotation, biology, genetics, and clinical feature analysis, combined with information in the pathogenic variant database, normal human genome database, and clinical feature database of 4000 known genetic diseases, the genetic data analysis algorithm classifies hundreds of thousands of genetic mutations. The pathogenicity of the variation was evaluated according to ACMG guidelines (Richards et al. 2015).

Sanger Sequencing

The candidate pathogenic genes identified by WES were confirmed by Sanger sequencing and cosegregation analyses among families. The PCR products were sequenced by ABI 3730XL DNA Sequencer (Applied Biosystems, Thermo Fisher Scientific, USA).

Results

Clinical Description

The proband was the first child of a nonconsanguineous couple (G1P1) without birth history of asphyxia and hypoxia, but her mother had a history of pregnancy preservation due to reduced fetal movement. She was diagnosed with growth retardation at 10 months, and she could communicate simply (no more than 3 words), walk upright, and run with a little difficulty at present after regular rehabilitation treatment. She presented with atonic seizures at 4 years old with low frequency and mild symptoms. After 6 years of age, the patients began to have recurrent seizures. Neurological examination demonstrated ataxia and intention tremor without any pathogenic reflex and increased deep tendon reflex. Electroencephalogram (EEG) showed pathogenic waves and brain magnetic resonance imaging (MRI) showed cerebellar tonsillar hernia (Fig. 1). She was given sodium valproate to control seizures and responded well to this therapy. To date, she has had almost no relapse since follow-up. Detailed clinical data are shown in Table 1.

Mutation Analysis

A variant in SEMA6B (NM_032108.4), c.1960_1978del/p. Leu654Argfs*25 (Fig. 2), was identified by whole-exome-sequencing in this patient. The variant was found to be a de novo variant without any family history of EPM-11 (the patient’s biological parents’ samples tested negative), which has not been reported in the literature. The variant is located in the last exon of SEMA6B and the phenotype of our patient also matches the clinical manifestations of EPM-11. PVS1 and PS2 should be strongly pathogenic in accordance with ACMG guidelines (Richards et al. 2015), and the minor allele frequency (MAF) was less than 0.0005; thus, the mutation incidence in this disease is expected to be rare and provides moderate evidence that it is a pathogenic mutation in EPM-11.
The SEMA6B gene contains 17 coding exons and a PPAR-binding site in the upstream sequence, and it is located on chromosome 19p13 (Correa et al. 2001). Through the use of immunostaining, Hamanaka et al. localized the expression of the SEMA6B gene to neurons in multiple brain regions, including the cerebral cortex, cerebellar Purkinje cells, and interneurons; specific cell types included excitatory and GABAergic inhibitory neurons (Hamanaka et al. 2020).

SEMA6B is a member of the class-6 semaphorin family, which is involved in different aspects of neural development, including neural crest cell migration, axon guidance, and cerebellar development (Andermatt et al. 2014). For example, Sema6B and Sema6A interact with PLXNA2 and plxna4 in axonal guidance in the regulation of mossy fiber targeting in the hippocampus (Suto et al. 2007; Tawarayama et al. 2010). Irwin Andermatt et al. found that Sema6B mediated the outgrowth response of commissural neurons by interacting with PlxnA2 and plxna4 in axonal guidance in the regulation of mossy fiber targeting in the hippocampus (Suto et al. 2007; Tawarayama et al. 2010).

Variants in other regions of the SEMA6B gene were found in the gnomAD database, but not in the last exon of the SEMA6B gene and rarely reported. Based on RNA analysis of lymphoblasts from a patient and experimental studies from animal model organisms, Hamanaka et al. speculated that these variants would lead to dominant-negative or gain-of-function effects rather than haploinsufficiency (Hamanaka et al. 2020). However, the variant of our patient was thought to be a loss-of-function variant in the NMD(-) region, which was located in the last 50 bp of the penultimate exon or in the last exon of the SEMA6B gene. Pathogenic variants related to EPM-11 were concentrated in exon 17 of SEMA6B, which was consistent with the distribution of pathogenic variants detected in our patients, indicating that exon 17 of SEMA6B is more clinically relevant.

The clinical presentations of all reported patients are summarized in Table 1. The manifestations are mainly characterized by common symptoms including developmental delay, seizures, intellectual disability, motor disturbance, ataxia, intention tremor, myoclonus, and abnormal EEG. Not all patients suffer from the following symptoms, such as microcephaly, rigidity, spasticity, increased deep tendon reflex, cerebellar atrophy, positive pathogenic reflexes, and cerebellar atrophy in brain MRI (Hamanaka et al. 2020). The condition of our patient was basically consistent with these
### Table 1  Clinical features of the patient reported in this work and comparison with published cases with SEMA6B-related progressive myoclonic epilepsy (Hamanaka et al. 2020)

| Clinical data | Kohei Hamanaka, et al (Individual 1) | Kohei Hamanaka, et al (Individual 2) | Kohei Hamanaka, et al (Individual 3) | Kohei Hamanaka, et al (Individual 4) | This case |
|---------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|-----------|
| Country       | Japanese                             | Japanese                             | Israeli                              | Malaysian                            | Chinese   |
| Current age   | 22 years                             | 28 years                             | 14 years                             | 11 years                             | 6 years   |
| Sex           | Male                                 | Female                               | Male                                 | Female                               | Female    |
| Locus         | SEMA6B (exon.17)                     | SEMA6B (exon.17)                     | SEMA6B (exon.17)                     | SEMA6B (exon.17)                     | SEMA6B (exon.17) |
| Pathogenic variant | c.1950_1969dup(p.Arg-657Profs*35) De novo | c.1976_1982del(p.Ala659Valfs*24) De novo | c.1991del(p.Gly664Alafs*21) De novo | c.1991del(p.Gly664Alafs*21) De novo | c.1960_1978del(p.Leu654Argfs*25) De novo |
| Age of onset  | 6 years                              | 11 months                            | 2 years                              | 4 years                              | 4 years   |
| Initial symptom | Developmental delay and seizure       | Seizure                              | Seizure                              | Developmental delay and seizure      | Developmental delay and seizure |
| Developmental history | Rolling over: 12 months; meaningful words: 24–36 months; initial walking without support: 17 months | Initial walking without support: 28 months | Initial walking without support: 24 months | Eye pursuit: 5 months; initial walking without support: 24 months; meaningful words: 30 months |
| Intellectual disability | Severe (IQ = 25 at 17 years) | Severe (IQ = 25 at 12 years) | Severe | Severe | Y (details not confirmed) |
| Microcephaly  | N                                    | Y (−2.0 SD)                          | Y (motor skill and dysarthria)      | Y (motor skill)                      | Y         |
| Regression    | Y (motor skill and dysarthria)       | Y (−2.5 SD)                          | Y (motor and verbal skills)         | Y                                    | N         |
| Seizure type at onset | GTCS since 6 years; absence seizures since 9 years; atomic seizures since 11 years | GTCS since 11 months; loss of consciousness with abnormal eye movement since 5 years; complex partial seizures since 10 years; atomic seizures since 10 years | Absence seizures since 2 years | Atomic seizures since 4 years | Atomic seizures since 4 years |
| Response to therapy (antiepileptic drugs) | Intractable | Intractable | Responsive | Intractable, but improved by clobazam and sulthiame | Responsive |
| Ataxia        | Y                                    | Y                                    | Y                                    | Y                                    | Y         |
| Intention tremor | Y                                   | Y                                    | Y                                    | Y                                    | Y         |
| Rigidity      | Y                                    | Y                                    | NP                                   | NP                                   | N         |
| Myoclonus     | Y                                    | Y                                    | NP                                   | NP                                   | N         |
| Spasticity    | Y                                    | Y                                    | NP                                   | NP                                   | N         |
| Increased deep tendon reflex | Y (upper and lower limbs) | Y (upper and lower limbs) | NP | N | N |
| Pathogenic reflex | Y (Rossolimo sign: positive, Mendel-Bechterew sign: positive) | Y (Rossolimo sign: positive, Mendel-Bechterew sign: positive) | Y (Rossolimo sign: positive, Mendel-Bechterew sign: positive) | Y (Rossolimo sign: positive, Mendel-Bechterew sign: positive) | Y (Rossolimo sign: positive, Mendel-Bechterew sign: positive) |
| Dystrophic features | N | N | N | N | N |
| Clinical data | Motor disturbance | Brain MRI | EEG | SEP | Other findings |
|---------------|-------------------|-----------|-----|-----|---------------|
| Kohei Hamanaka, et al (Individual 1) | Wheelchair | Normal | Abnormal discharge in right hemisphere (6 years); burst of diffuse irregular spikes and slow waves (9 years); diffuse spike and slow waves in frontal, parietal, and temporal regions (14 years) | Prolonged N20 latency and high amplitude of P24-N33 | NP |
| Kohei Hamanaka, et al (Individual 2) | Wheelchair | Mild cerebellar atrophy | Diffuse slow wave with 2–3 Hz and spike-and-wave in bilateral frontal region (3 years and 4 years); diffuse theta waves with 4–5 Hz and spike-and-wave burst with 2–3 Hz (9 years); multifocal spikes in left parietal region and bilateral frontal regions (12 years); multifocal in left occipital region (13 years); slow waves at baselines (23 years) | Giant SEP | SLE |
| Kohei Hamanaka, et al (Individual 3) | Wheelchair | Small vermis | Abnormal background activity (1 year); slow abnormal sleep features with a paucity of sleep spindles (13 years) | NP | NP |
| Kohei Hamanaka, et al (Individual 4) | Walking with support | Normal | Focal bifrontal epileptiform discharges accentuated during sleep (4 years); frequent frontocentral discharges during awake state (5 years); frequent intermittent slow spikes in right posterior region (11 years) | NP | NP |
| This case | Running and jumping with a little difficulty | Herniation of the cerebellar tonsils | Diffuse spike-and-wave burst with 1.5–2.5 Hz, especially in frontal regions; temporary increase in myoelectric activity; interictal widespread slow spina wave with 1.5–2.0 Hz released continuously and irregularly (6 years) | U | U |

*EEG electroencephalogram, GTCS generalized tonic–clonic seizures, MRI magnetic resonance imaging, SEP somatosensory evoked potential, SLE systemic lupus erythematosus, NP not performed, U unknown, Y present, N not present*
common clinical features, lack of microcephaly, and cerebellar atrophy. Interestingly, her brain MRI demonstrated cerebellar tonsils herniation rather than cerebellar atrophy, whose link to the EPM-11 has not been confirmed. We may obtain more complete understanding of this disease via the study of these rare cases, which could also help in the differential diagnosis of EPM-11.

All reported cases and this case exhibited the clinical features of EPM-11, and their pathogenic variants were all located in exon 17 of SEMA6B (Fig. 3). To further verify its specificity for EPM-11, Hamanaka et al. created truncating mutations in exon 17 of SEMA6B and loss-of-function mutations in exon 2 of SEMA6B by injecting CRISPR RNAs (crRNAs) to zebrafish models (Hamanaka et al. 2020). It turned out that zebrafish injected by SEMA6B-exon 17 crRNAs demonstrated increased hyperactive responses to epileptogenic stimuli and more severe developmental defects with CNS anomalies compared with the response of the SEMA6B-exon 2-injected zebrafish (Hamanaka et al. 2020). What is more, a missense mutation in exon 16 of SEMA6B
A/p.Val612Met, was found to be possibly related to cerebellar hypoplasia, whose symptoms included cerebellar atrophy and global developmental delay, but whether the mutation was pathogenic has not been confirmed (Aldinger et al. 2019). These findings suggested that truncating mutations occurred within exon 17 of SEMA6B were more clinically associated with EPM-11 compared with variants in other regions of the SEMA6B gene.

**Conclusions**

We reported the first EPM-11 case with a de novo heterozygous frameshift mutation in China. The new genetic evidence we reported here strengthened the gene-disease relationship, because the gene curation level between SEMA6B and EPM-11 became “strong” following ClinGen SOP. This finding revealed the role of new mutations as important genetic causes of EPM-11 and broadened the molecular genetic basis of EPM-11.

**Abbreviations** SEMA6B: Semaphorin 6B; MRI: Magnetic resonance imaging; EPM: Progressive myoclonic epilepsy; EEG: Electroencephalogram

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**Author Contribution** QL and ML drafted the manuscript and contributed to conception and design of the study. DPH, PJ, and WHL cared for the patient and critically revised the article. JH and TL contributed to the data acquisition. XQC critically revised and gave final approval for publication of the paper.

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**Data Availability** The data and material discussed here are available in the references listed.

**Declarations**

**Ethics Approval and Consent to Participate** The study was based on the principles of the Declaration of Helsinki and authorized by the Institutional Review Committee of Children’s Hospital Affiliated to Soochow University. Written consent for publication was obtained from the patient.

**Consent for Publication** All participants signed written informed consent forms.

**Conflict of Interest** The authors declare no competing interests.

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