Original Article

Identification of a Novel Homozygous Splice-Site Mutation in *SCARB2* that Causes Progressive Myoclonus Epilepsy with or without Renal Failure

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Abstract

Background: Progressive myoclonus epilepsies (PMEs) comprise a group of rare genetic disorders characterized by action myoclonus, epileptic seizures, and ataxia with progressive neurologic decline. Due to clinical and genetic heterogeneity of PMEs, it is difficult to decide which genes are affected. The aim of this study was to report an action myoclonus with or without renal failure syndrome (EPM4) family and summarize the clinical and genetic characteristics of all reported EPM4 patients.

Methods: In the present study, targeted next-generation sequencing (NGS) was applied to screen causative genes in a Chinese PME family. The candidate variant was further confirmed by cosegregation analysis and further functional analysis, including the reverse transcription polymerase chain reaction and Western blot of the proband's muscle. Moreover, literature data on the clinical and mutational features of all reported EPM4 patients were reviewed.

Results: The gene analysis revealed a novel homozygous splicing mutation (c.995-1G>A) of the *SCARB2* gene in two brothers. Further functional analysis revealed that this mutation led to loss function of the *SCARB2* protein. The classification of the candidate variant, according to the American College of Medical Genetics and Genomics standards and guidelines and functional analysis, was pathogenic. Therefore, these two brothers were finally diagnostically confirmed as EPM4.

Conclusions: These present results suggest the potential for targeted NGS to conduct a more rapid and precise diagnosis for PME patients. A literature review revealed that mutations in the different functional domains of *SCARB2* appear to be associated with the phenotype of EPM4.

Key words: Progressive Myoclonus Epilepsies; Progressive Myoclonus Epilepsy with or without Renal Failure; *SCARB2* Gene; Targeted Next-Generation Sequencing

Introduction

Progressive myoclonus epilepsies (PMEs) comprise a group of hereditary disorders characterized by action myoclonus, epileptic seizures, and ataxia with progressive neurologic decline.[1] Owing to clinical and molecular heterogeneity, PMEs present with various forms caused by different disease-causing genes. According to the Online Mendelian Inheritance in Man database and relevant literature, PMEs can be divided into 12 subtypes: Unverricht–Lundborg disease (EPM1A), EPM1B, Lafora body disease (EPM2A), Lafora body disease (EPM2B), EPM3, action myoclonus with or without renal failure syndrome (EPM4), PME-ataxia syndrome (EPM5), North Sea PME (EPM6), EPM7, EPM8, EPM9, and EPM10. The respective disease-causative genes are as follows: *CSTB*, *PRICKLE1*, *MELF*, *NHLRC1*, *KCTD7*, *SCARB2*, *PRICKLE2*, *GOSR2*, *KCNC1*, *CERS1*, *LMNB2*, and *PRDM8*.[1-13] However, there are other neurogenetic diseases mainly characterized by myoclonus, epileptic seizures, and ataxia, such as myoclonus epilepsy and ragged red fibers, neuronal ceroid lipofuscinoses, sialidosis, dentatorubral-pallidoluysian atrophy (DRPLA),

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and neuronopathic Gaucher disease, while a literature also regarded these diseases as PMEs.[1] In clinic, it is difficult to make an exact diagnosis among the various forms of PMEs due to homogeneous phenotypes. Moreover, other diseases, such as juvenile myoclonic epilepsy, inherited ataxia, and mitochondrial disease, also resemble or overlap PMEs in clinical features, which present challenges in the differential diagnosis. Therefore, the screening for the disease-related mutation of pathogenic genes is particularly valuable in the diagnosis of PMEs. Traditional Sanger sequencing lacks the efficiency to handle larger numbers of candidate genes associated with PMEs. As a powerful approach for genetic diagnostics in inherited Mendelian disorders, targeted next-generation sequencing (NGS) has increased the ability to rapidly and effectively sequence any genomic region of interest.[14,15]

In the present study, two brothers were confirmed as EPM4 with the application of a targeted NGS panel, which covers the causative genes of PMEs, juvenile myoclonic epilepsy,[16] inherited ataxia, and mitochondrial disease. The investigators found a novel splice mutation of scavenger receptor class B, member 2 (*SCARB2*), and further validated the candidate variant by functional research based on the patient's tissues. Furthermore, literature data were reviewed to summarize the clinical and mutational spectrum of all reported EPM4 patients worldwide.

Methods

Ethical approval

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University. Written informed consent was obtained from all participants in the family and individuals in the control group.

Subject

A family from Fujian Province, China, who presented with a PME phenotype, was recruited in the present study. Detailed clinical data, including clinical presentations, physical examinations, laboratory tests, and neuroimaging and electroencephalography (EEG) results, were collected. Three hundred unrelated healthy individuals with no known history of neurogenetic disease were collected and assigned as the control group.

Genetic testing of dentatorubral-pallidoluysian atrophy

For the purpose of excluding DRPLA, the patient was tested for CAG trinucleotide repeats in the *ATN1* gene of the proband by polymerase chain reaction (PCR), as previously described.[17]

Targeted next-generation sequencing and sequence analysis

Targeted NGS was performed on genomic DNA samples extracted from the proband and his elder brother's peripheral blood samples using a Blood Genomic Extraction Kit (Qiagen, Hilden, Germany). The sequences were performed by an NGS-based assay using the Illumina HiSeq2500 platform (Illumina, California, USA). The panel was prepared using a NimbleGen SeqCap EZ Choice kit (Roche, Basel, Switzerland), which included 927 disease-causative genes of the neurogenetic disease, containing progressive myoclonic epilepsy, hereditary ataxia, mitochondrial diseases, neuronal ceroid lipofuscinosis and other neurogenetic diseases. Targeted coding exons and intron-exon regions corresponded to 4.8 Mb of the genomic sequence.

First, the raw sequence reads were aligned to the human reference genome (UCSC hg 19) (http://hgdownload.cse.ucsc.edu/) using Burrows–Wheeler Aligner (Li and Durbin, 2009). Second, the gene-, region-, and filter-based levels of the variants were annotated using the ANNOVAR software (version Feb 11, 2013, GitHub, Philadelphia, USA). Then, the frequency of the variants was further determined using the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genomes Project (http://ftp.ncbi.nih.gov/), and the Exome Aggregation Consortium (http://exac.broadinstitute.org/). Finally, the filtered variants were classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines.

Sanger sequencing

Sanger sequencing was further performed to verify the candidate variants, and cosegregation analysis was performed among family members. The candidate regions were amplified by PCR and sequenced using the ABI PRISM 3730 gene analyzer (Applied Biosystems, California, USA).

Reverse transcriptase polymerase chain reaction

To evaluate whether the candidate variants influence the expression of mRNA, total RNA was isolated from frozen muscle treated with TRIzol Reagent (Life Technologies, California, USA), and single-strand cDNA was prepared using the PrimeScript® RTase Kit (Takara, Otsu, Japan). The candidate region of *SCARB2* was amplified and sequenced using the following primers: Forward: 5'-TGACTATGAGAGTGTACAGG-3'; Reverse: 5'-TGGTCTTCCTGATTTGGGTG-3'.

Western blot

Protein was isolated from frozen muscle treated with RIPA and PMSF (Beyotime, Shanghai, China). Thirty microgram of total protein was resolved on 10% SDS-PAGE gels, and the proteins were transferred onto nitrocellulose (NC) membranes. Then, the NC membranes were blocked with 5% nonfat milk in Tris-buffered saline with Tween solution (0.01 mol/L of Tris HCl, 0.15 mol/L NaCl, and 0.1% Tween) and probed with the appropriate primary antibody (mouse anti-LIMP2 antibody, Santa Cruz Biotechnology, USA; mouse anti-glyceraldehyde phosphate dehydrogenase antibody, Beyotime, China). Next, the membranes were incubated with the appropriate peroxidase-labeled mouse anti-goat Ig and developed with enhanced chemiluminescent detection reagents (Beyotime, Shanghai, China).

Results

Clinical features of the family

The proband is a 23-year-old male. He began to experience myoclonic jerks of the upper limbs and shoulders at the age of 21, especially when he felt nervous or fell asleep. Five episodes of generalized tonic–clonic seizures occurred 2 years later without any medication. On examination, his intelligence was normal. There was prominent action myoclonus that involved the upper limbs and shoulders. The other neurologic examination revealed cerebellar features, including dysarthria, a broad-based gait, abnormal heel–knee–tibia test, and finger–nose test. Mild generalized skeletal muscle atrophy without fasciculations and *pes cavus* was also observed.

The routine blood biochemical test results, including normal serum blood urea nitrogen, creatinine, and urinalysis, were unremarkable. EEG revealed multifocal spike and wave complexes, especially in the left parietal lobe, occipital lobe, and temporal lobe [Figure 1a]. Brain MRI revealed mild cerebellar atrophy [Figure 1b and 1c]. Muscle biopsy presented with certain muscle atrophy and other less obvious signs, but there were no ragged red fibers [Figure 1d].

The elder brother of the proband was found to have similar symptoms at the age of 20 years, with myoclonus, ataxia, and generalized tonic-clonic seizures. He started to experience anxiety and myoclonic jerks of the upper limbs and presented with three generalized episodes of tonic-clonic seizures and loss of consciousness in 1 year. After the medication of sodium valproate and lamotrigine, the symptom of epilepsy did not occur. The neurologic examination revealed ataxia and muscle weakness. This patient did not undergo blood biochemical tests, brain MRI, and muscle biopsy.

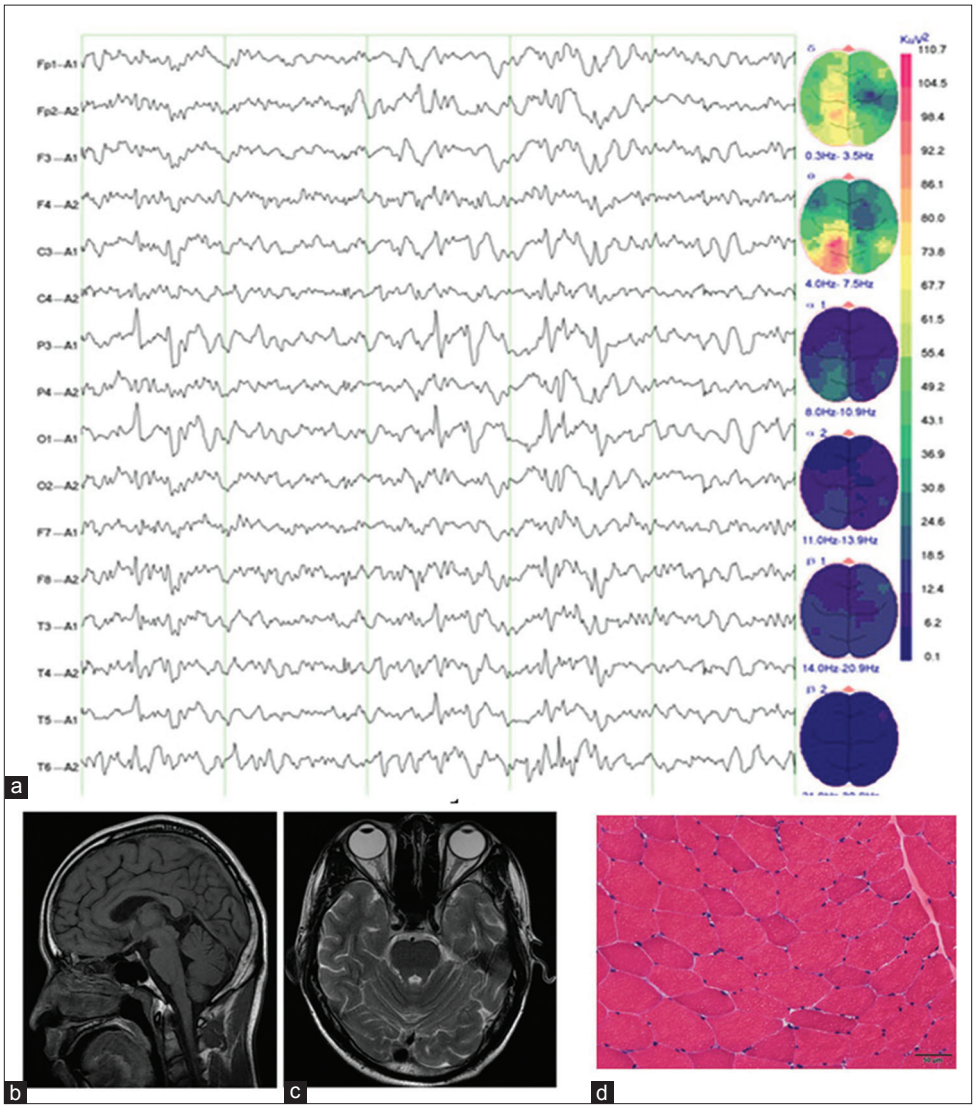

Figure 1: The auxiliary examinations of the proband. (a) EEG revealed multifocal spike and wave complexes, especially in the left parietal lobe, occipital lobe, and temporal lobe. (b) Axial brain MRI revealed mild cerebellar atrophy. (c) Transverse MRI scan revealed mild cerebellar atrophy. (d) Muscle biopsy presented with certain muscle atrophy and other less obvious signs (H and E, original magnification ×100). MRI: Magnetic resonance imaging; EEG: Electroencephalography.

Dentatorubral–pallidoluysian atrophy CAG trinucleotide repeats analysis

The PCR analysis demonstrated that the number of *ATN1* CAG repeats was within the normal range in the proband.

Identification of variants by targeted next-generation sequencing analysis

Targeted NGS was performed in the two patients. The coverage of the fraction of the target base is presented in Supplementary Table 1. The mean coverage of the target bases was 92.1698 (II1) and 111.7527 (II2), respectively. Furthermore, the total SNP variants, including SNPs, noncoding region variants, synonymous mutations, and missense mutations, were 3499 (II1) and 3082 (II2), respectively, while the total initial map of insertion and deletion (INDEL) variants, including insertion and deletion, were 161 (II1) and 180 (II2), respectively. Through the further bioinformatic analysis of these two patients, it was found that these patients harbored a splice-site homozygous mutation in the *SCARB2* gene (c.995-1G>A), which was an unreported splicing variant.

Sanger sequencing and cosegregation analysis

The *SCARB2* c.995-1G>A homozygous mutation identified by targeted NGS was further confirmed in the two brothers by Sanger sequencing [Figure 2]. The homozygous mutation was also found in his elder brother, and *SCARB2* c.995-1G>A was heterozygous in his unaffected parents [Figure 2]. Meanwhile, the mutation was not detected in the 300 unrelated controls. Therefore, the *SCARB2* c.995-1G>A homozygous mutation cosegregated with the PME family.

SCARB2 gene and protein expression analysis

To evaluate the meaning of the *SCARB2* c.995-1G>A homozygous mutation, the cDNA and protein levels of *SCARB2* were analyzed by RT-PCR and Western blot, respectively. After Sanger sequencing to the cDNA of the *SCARB2* gene, as presented in Figure 3a, a c.995-1036del42 mutation was observed in the proband. Furthermore, Western blot was performed to evaluate the expression and quality of the *SCARB2* protein. As presented in Figure 3b, a truncated *SCARB2* protein is observed, which is accordant with the c.995-1036del42 mutation in the cDNA of patients who harbored the *SCARB2*c.995-1G>A homozygous mutation. Compared to the full length of the 72,000 of the *SCARB2* protein in healthy controls, the expression of the truncated *SCARB2* protein that weighted from 43,000 to 55,000 significantly decreased ($t = 2.887$, $P = 0.0447$) [Figure 3b].

Discussion

In the present study, targeted NGS technique was applied to screen disease-causative genes in a PME family. A novel *SCARB2* splicing homozygous variant c.995-1G>A was identified in this family. According to the standards and guidelines of the ACMG, the variant was classified as a pathogenic variant. Further functional analysis confirmed that the c.995-1G>A variant can lead to the loss function of *SCARB2* protein. Therefore, the two brothers were finally diagnostically confirmed with EPM4.

EPM4 is a rare form of PMEs and is an autosomal recessive inherited disorder caused by homozygous mutation and compound heterozygous mutation in the *SCARB2* gene.[18,19] Due to the genetic heterogeneity and clinical variability of PMEs, it is often challenging to detect particular gene mutations by depending solely on phenotypes. In clinic, there is a need to establish a molecular diagnostic strategy for the screening of disease-causative genes in PME cases. Targeted NGS is a high-throughput and cost-effective method to screen genomic regions of interest. This approach has been applied for inherited Mendelian disorders.[14,15] Once the panel is established, it can be used for the same genomic region in different cases. In the present study, a panel that included 927 disease-causative genes of neurogenetic diseases was established and used for detecting the culprit genes of PMEs and other neurogenetic diseases. However, the disadvantage of targeted NGS is that the approach cannot accurately detect trinucleotide repeats and copy number variations. Hence,

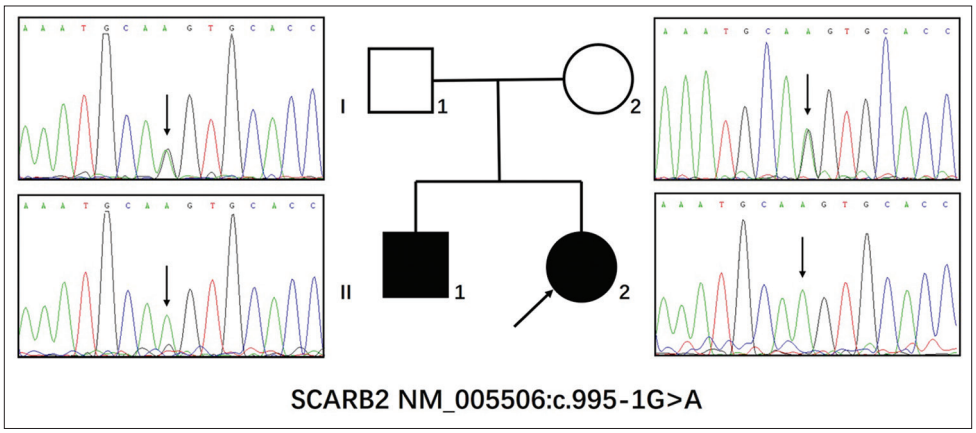

Figure 2: Sanger sequencing of the family with progressive myoclonus epilepsies. The two brothers were tested for the homozygous splice mutation (c.995-1G>A) of the *SCARB2* gene. Then, their parents were tested for heterozygous mutations of the *SCARB2* gene. The arrow indicated the homozygous splice mutation (c.995-1G>A).

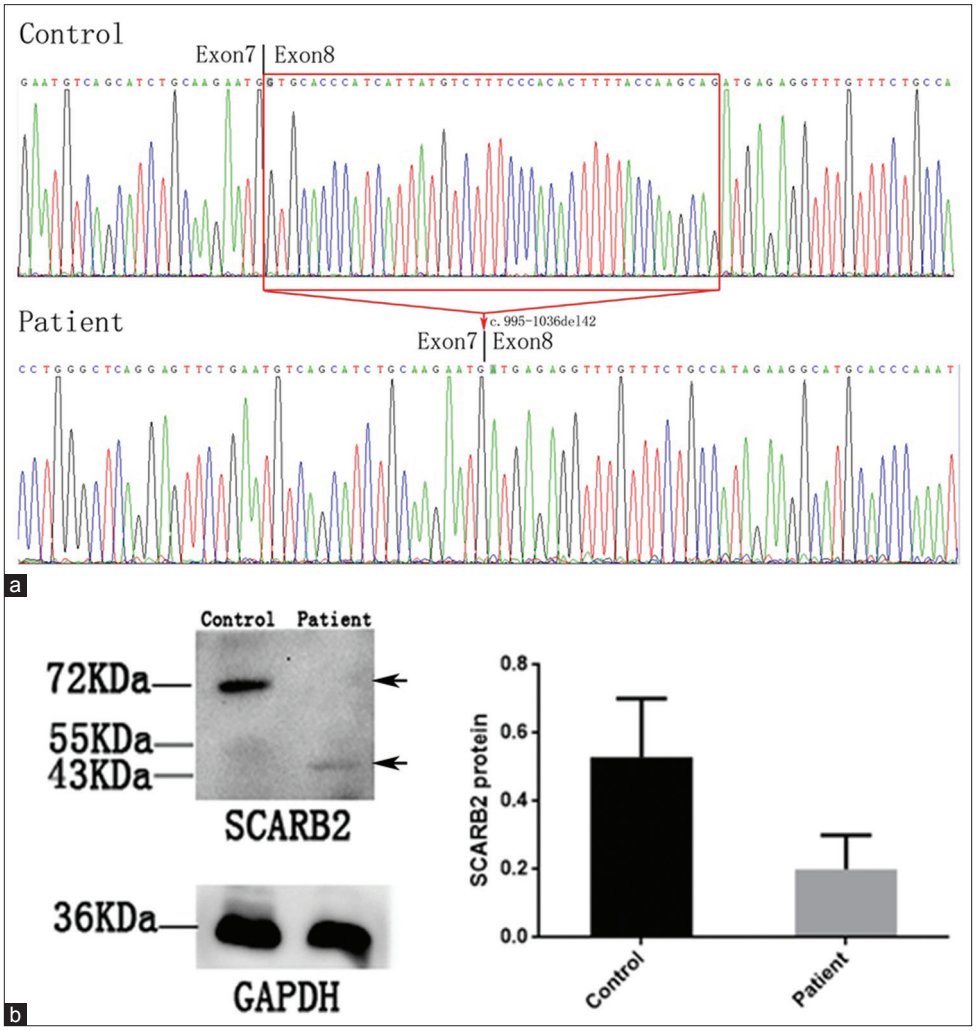

Figure 3: *SCARB2* gene expression and protein expression analysis of the proband. (a) Sanger sequencing to the cDNA of the *SCARB2* gene: a c. 995-1036del42 mutation was observed in the proband. (b) Western blot analysis of the protein obtained from the muscle of proband and controls. Compared to the full length of the 72,000 of SCARB2 protein in healthy controls, the expression of the truncated SCARB2 protein that weighted from 43,000 to 55,000 significantly decreased ($t = 2.887$, $P = 0.0447$). The arrow indicated a 43,000-protein band appeared in patients but a 72,000 band in control.

for PME cases, before applying targeted NGS, the CAG trinucleotide repeats of DRPLA needs to be initially screened.

To date, only few EPM4 patients have been recorded worldwide. The correlation of genotypes and phenotypes among EPM4 patients has seldom been summarized in previous literature. The studies conducted by the investigators identified both mutant alleles in the two brothers with EPM4, providing more information to further analyze the clinical features and mutational spectrum of all reported EPM4 patients [Table 1].[18-30] According to the literature review and the present study, the median age of onset is 20 years (range: 11–52 years) and the median age of death is 30.5 years (range: 23–59 years). All EPM4 patients begin with three typical manifestations: action myoclonus, generalized seizures, and ataxia. However, the median onset age of these three typical manifestations shows little statistical difference: action myoclonus presents at 21 years (range: 14–57 years), generalized seizure presents at 21.5 years (range: 16–63 years), and ataxia presents at 20.5 years (range: 14–58 years). With regard to renal failure, 11 patients suffered from this and developed EPM4 in adolescence (median: 17 years), while the other 17 patients had no renal dysfunction, but presented initial signs at a later age (median: 22 years) ($P = 0.033$). In addition, other malfunctions were also observed in previous studies: hearing loss occurred in two patients, cognitive decline occurred in two patients, and demyelinating polyneuropathy occurred in four patients.[22-25] In the present study, it was found that the proband and his brother presented with talipes cavus, which may suggest that they have acquired peripheral neuropathy.

The *SCARB2* gene encodes *SCARB2* protein in humans and is also known as lysosomal integral membrane protein type-2 (LIMP2), which is a disease causative of EPM4, and is associated with Parkinson's disease.[18,19,31,32] LIMP2, which is a nonspecifically expressed transmembrane (TM) protein, is mainly located in lysosomes and endosomes.[33] While bounding with beta-glucocerebrosidase (GBA), LIMP2 can transfer GBA from the endoplasmic reticulum to

Table 1: Literature data on the clinical and mutational features of all reported EPM4

Reference	Case	Mutation type	Exon/intron	Nucleotide mutation	Protein alteration	Location of mutation	Sex
Balreira *et al*. Hum Mol Genet[18]	1	Homozygote	Exon 4	c.533G>A	W178X	GBA binding domain	Female
	2	Homozygote	Exon 4	c.533G>A	W178X	GBA binding domain	Female
Berkovic *et al*. Am J Hum Genet[19]	3	Homozygote	Intron 10	c.1239+1G>T	N	CD36 like domain	Female
Berkovic *et al*. Am J Hum Genet[19]	4	Homozygote	Exon 4	c.435_436insAG	W146SfsX16	GBA binding domain	Female
Berkovic *et al*. Am J Hum Genet[19]	5	Compound heterozygote	Exon 3	c.296 delA	N99IfsX34	CD36 like domain	Male
			Intron 5	c.704+5G>A	N	GBA binding domain	
Berkovic *et al*. Am J Hum Genet[19]	6	Homozygote	Exon 7	c.862C>T	Q288X	GBA binding domain	–
Dardis *et al*. Mol Genet Metab[20]	7	Homozygote	Exon 8	c.1087C>A	H363N	CD36 like domain	Female
Dibbens *et al*. Ann Neurol[21]	8	Homozygote	Intron 8	c.1116-2A>C	N	CD36 like domain	Male
Dibbens *et al*. Ann Neurol[21]	9	Homozygote	Intron 5	c.704+1G>C	N	GBA binding domain	Male
Dibbens *et al*. Ann Neurol[21]	10	Homozygote	Exon 11	c.1258delG	E420RfsX5	CD36 like domain	Female
Dibbens *et al*. Ann Neurol[21]	11	Homozygote	Exon 5	c.666delCCTTA	Y222X	GBA binding domain	Female
Dibbens *et al*. Ann Neurol[21]	12	Compound heterozygote	Intron 3	c.424-2A>C	N	CD36 like domain	Female
			Exon 8	c.1087C>A	H363N	CD36 like domain	
Dibbens *et al*. Arch Neurol[22]	13	Compound heterozygote	Exon 7	c.862C>T	Q288X	GBA binding domain	Male
			Intron 9	c.1187+3insT	N	CD36 like domain	
Hopfner *et al*. BMC Neurol[23]	14	Homozygote	Exon 1	c.111delC	I37MfsX7	CD36 like domain	Male
	15	Homozygote	Exon 1	c.111delC	I37MfsX7	CD36 like domain	Male
	16	Homozygote	Exon 1	c.111delC	I37MfsX7	CD36 like domain	Female
Perandones *et al*. Mov Disord[24]	17	Homozygote	Intron 5	c.704+1G>A	N	GBA binding domain	Female
Guerrero-López *et al*. Mov Disord[28]	18	Homozygote	Exon 8	c.1015insT	F339FfsX9	CD36 like domain	Female
Higashiyama *et al*. Mov Disord[29]	19	Homozygote	Exon 11	c.1385_1390del6insATGCATGCACC	G462DfsX34	TM domain	Female
	20	Homozygote	Exon 11	c.1385_1390del6insATGCATGCACC	G462DfsX34	TM domain	Male
Fu *et al*. Neuropathol Appl Neurobiol[25]	21	Homozygote	Exon 11	c.1385_1390del6insATGCATGCACC	G462DfsX34	TM domain	Male
Fu *et al*. Neuropathol Appl Neurobiol[25]	22	Homozygote	Exon 3	c.361C>T	R121X	CD36 like domain	Female
Zeigler *et al*. J Neurol Sci[27]	23	Homozygote	Exon 11	c.1270C>T	R424X	CD36 like domain	Male
	24	Homozygote	Exon 11	c.1270C>T	R424X	CD36 like domain	Female
He *et al*. Clin Genet[26]	25	Homozygote	Exon 11	c.1270C>T	R424X	CD36 like domain	Female
	26	Homozygote	Exon 11	c.1270C>T	R424X	CD36 like domain	Female
This study	27	Homozygote	Exon 7	c.995-1G>A	N	CD36 like domain	Male
	28	Homozygote	Exon 7	c.995-1G>A	N	CD36 like domain	Male

Reference	Age of onset (years)	Age at death (years)	Action myoclonus (years)	Tonic clonic seizures (years)	Ataxia (years)	Renal failure (years)	Phenotype	Other specific phenotype
Balreira *et al*. Hum Mol Genet[18]	15	23	15	–	18	18	With renal failure	N
	17	26	15	–	17	21	With renal failure	N
Berkovic *et al*. Am J Hum Genet[19]	11	–	–	–	–	–	With renal failure	N
Berkovic *et al*. Am J Hum Genet[19]	–	–	–	–	–	–	With renal failure	N
Berkovic *et al*. Am J Hum Genet[19]	11	–	–	–	–	–	With renal failure	N
Berkovic *et al*. Am J Hum Genet[19]	–	–	–	–	–	–	With renal failure	N

Contd...

Table 1: Contd...

Reference	Age of onset (years)	Age at death (years)	Action myoclonus (years)	Tonic clonic seizures (years)	Ataxia (years)	Renal failure (years)	Phenotype	Other specific phenotype
Dardis *et al*. Mol Genet Metab[20]	26	–	26	27	26	N	Without renal failure	N
Dibbens *et al*. Ann Neurol[21]	14	29	14	17	17	N	Without renal failure	N
Dibbens *et al*. Ann Neurol[21]	15	27	15	16	16	N	Without renal failure	N
Dibbens *et al*. Ann Neurol[21]	23	33	23	23	24	N	Without renal failure	N
Dibbens *et al*. Ann Neurol[21]	25	40	25	28	31	N	Without renal failure	N
Dibbens *et al*. Ann Neurol[21]	26	32	26	26	27.5	N	Without renal failure	N
Dibbens *et al*. Arch Neurol[22]	16	–	16	20	20	N	Without renal failure	Demyelinating polyneuropathy
Hopfner *et al*. BMC Neurol[23]	14	31	14	20	14	–	With renal failure	Demyelinating polyneuropathy
	20	38	26	32	20	–	With renal failure	Hearing loss, demyelinating polyneuropathy
	20	34	20	20	20	–	With renal failure	Demyelinating polyneuropathy
Perandones *et al*. Mov Disord[24]	21	–	23	25	21	25	With renal failure	Hearing loss
Guerrero-López *et al*. Mov Disord[28]	22	–	22	22	30	N	Without renal failure	N
Higashiyama *et al*. Mov Disord[29]	43	–	43	58	58	N	Without renal failure	N
	52	–	57	63	52	N	Without renal failure	Acute ischemic stroke
Fu *et al*. Neuropathol Appl Neurobiol[25]	45	59	48	–	51	N	Without renal failure	Dementia
Fu *et al*. Neuropathol Appl Neurobiol[25]	20	28	20	20	20	N	Without renal failure	Cognitive decline
Zeigler *et al*. J Neurol Sci[27]	17	30	17	17	17	29	With renal failure	N
	17	27	17	17	17	N	Without renal failure	
He *et al*. Clin Genet[26]	21	–	21	25	22	N	Without renal failure	N
	27	–	27	N	27	N	Without renal failure	
This study	21	–	21	21	21	N	Without renal failure	Talipes cavus
	20	–	20	20	20	N	Without renal failure	Talipes cavus

–: Not mention in the literature; N: Do not have the symptom; GBA: Beta-glucocerebrosidase; TM: Transmembrane.

the lysosome.[34-39] *SCARB2* protein comprises two TM domains and one CD36-like domain, which contains a highly conserved coiled-coil domain (residue: 145-288 aa) that binds GBA [Figure 4].[35-37] Mutations in the *SCARB2* gene result in decreasing and anomaly location of *SCARB2* protein, affecting the combination of SCARB and GBA.

The genotype and phenotype correlation of all reported EPM4 patients and the two brothers, including the 19 *SCARB2* gene mutations in 28 EPM4 patients, is summarized in the present study [Table 1 and Figure 4].[18-30] Among these 19 mutations, 16 mutations (84.21%) were homozygous mutations, while only 3 mutations were compound heterozygous. The mutation types of *SCARB2* were nonsense, frameshift, and splice-site mutations, which can be assumed to disrupt gene function, leading to the complete absence of the gene product through the lack of transcription or nonsense-mediated decay of the altered transcript. For the phenotype with renal failure, seven mutations were found in 11 patients, of which the four mutations located in the GBA-binding domain may directly disrupt *SCARB2* bounding with GBA. Merely two mutations were not located in the GBA-binding domain. Although the I37MfsX7 homozygous mutation was located in this domain, it was a frameshift mutation that may produce a truncated protein with no GBA-binding domain. For the phenotype without renal failure, there were 11 homozygous mutations. Among these, eight mutations were located after the GBA-binding domain, which may not affect the combination of GBA and *SCARB2*, while two mutations were compound heterozygous mutations. Merely one mutation was located in the TM domain, which led to a late-onset phenotype.[25,29] These data possibly reveal that the functional domains of the *SCARB2* gene are associated with the EPM4 phenotype. Interestingly, there was a marked variability of clinical features between these two patients in EPM4 families (family 17) with the same nucleotide position (R424X), and the variability was also found in family 14 (c.704+1G>A) though follow-up studies.[27,30] Hence, it could be speculated that the phenotypic difference may be due to genetic modifiers or environmental factors. Therefore, more data need to be acquired before a specific phenotype–genotype correlation could be determined.

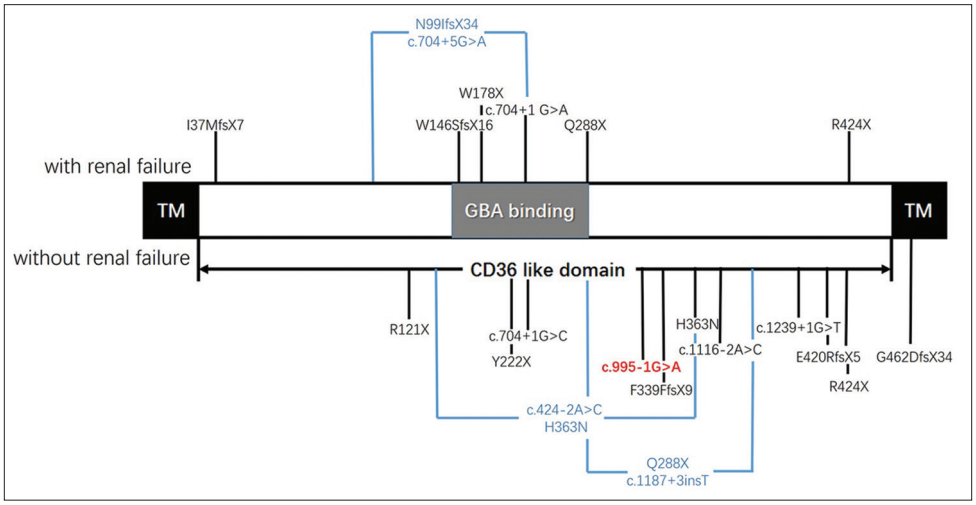

Figure 4: The structure of the *SCARB2* gene and the reported mutation of SCARB2. GBA: Beta-glucocerebrosidase; TM: Transmembrane.

In conclusion, the present study reported two EPM4 brothers with a novel splice mutation in the *SCARB2* gene detected by targeted NGS analysis. A literature review revealed that pathogenic mutations of the *SCARB2* gene in EPM4 patients are homozygous mutations rather than compound heterozygous mutations. The mutations in the different functional domains of *SCARB2* appear to be associated with the phenotype of EPM4.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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SCARB2基因新剪接位点纯合突变导致进行性肌阵挛癫痫伴或不伴肾功能衰竭

摘要

背景：进行性癫痫性肌阵挛（PMEs）是一组以肌阵挛、癫痫发作、共济失调及神经系统功能进行性减退为特点的罕见病。由于该病具有临床和基因异质性，明确疾病的致病基因有一定难度。 本文对一例EPM4家系进行了报道并总结了所有已报道EMP4患者的临床与基因突变特点。

方法：本研究应用目标区域测序在一个家系中检测 PMEs致病基因并对候选基因变异位点进行家系共分离分析以及对先证者肌肉细胞进行基因表达水平和蛋白表达水平分析。同时，回顾并总结所有已报道EPM4临床和突变特点的文献。

结果：通过基因分析发现，先证者及其兄长均携带SCARB2基因c.995-1G>A剪切位点突变。进一步实验发现该突变导致SCARB2蛋白功能缺失。根据美国医学遗传学和基因组学学院（ACMG）标准和指南，该候选变异位点为致病突变，该兄弟最终被确诊EPM4。

结论：靶向二代测序未来有望成为更加快速精准诊断PMEs的方法。通过回顾相关文献表明EPM4的表型与突变位点所在SCARB2基因功能域有关。

Supplementary Table 1: The coverage of the fraction of target base of II1 and II2

Items	II1	II2
Total read	5,637,906	6,396,240
Total mapped read	5,575,859	6,311,962
Unique mapped	5,255,824	5,982,202
No-mismatch mapped	3,509,971	4,152,867
Mismatch alignment bases rate	0.3774	0.3507
Reads on target regions	4,037,140	4,883,548
Fraction on target regions	0.7161	0.7635
Fraction on target regions covered by reads	0.9982	0.9987
Unique mapped reads on target regions	3,832,984	4,658,405
No-mismatch reads on target regions	2,577,212	3,232,079
Reads on target ± 150 regions	4,652,214	5,360,719
Fraction on target ± 150 regions	0.8252	0.8381
Fraction on target ± 150 regions covered by reads	0.996	0.9961
Unique mapped reads on target ± 150 regions	4,407,479	5,100,383
No-mismatch reads on target ± 150 regions	2,937,340	3,524,865
Reads on target ± 500 regions	4,801,824	5,411,318
Fraction on target ± 500 regions	0.8517	0.846
Fraction on target ± 500 regions covered by reads	0.8957	0.8004
Unique mapped reads on target ± 500 regions	4,542,903	5,145,900
No-mismatch reads on target ± 500 regions	3,011,736	3,551,922
Fraction of target bases covered	0.9982	0.9987
Fraction of target bases covered with 0~5X	0.012	0.0071
Fraction of target bases covered with 5~10X	0.0171	0.0099
Fraction of target bases covered with 10~15X	0.0207	0.0122
Fraction of target bases covered with 15~20X	0.0257	0.0138
Fraction of target bases covered with 20~25X	0.0316	0.0157
Fraction of target bases covered with 25~30X	0.0355	0.0179
Fraction of target bases covered with 30~35X	0.0382	0.0222
Fraction of target bases covered with 35~40X	0.0417	0.0252
Fraction of target bases covered with 40~45X	0.0439	0.0287
Fraction of target bases covered with 45~50X	0.0452	0.0308
Fraction of target bases covered with >50X	0.6865	0.8152
Mean Coverage of target bases	92.1698	111.7527