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Novel compound heterozygous mutations in LMAN2L cause early childhood refractory epilepsy

Teng Wang[1], Yan Gao[1], Yuhan Yan[1], Ping Yin[1], Lili Tong[1] and Meng Dong[1*]

Abstract

Background Autosomal recessive mental retardation-52(MRT52) is a subtype of mental retardation whose clinical features include global developmental delay, severe intellectual disability with poor speech, and mild seizures in early childhood. Mutations in the *LMAN2L* gene resulting in mental retardation and seizures have been previously reported in 3 families. Here we describe 2 children in 1 family who presented with severe intellectual disability and drug-refractory epilepsy(DRE) at 2 months of age.

Methods Two individuals from 1 family admitted to the pediatric department of Qilu Hospital were included in the study. Whole exome sequencing (WES) was used to detect *LMAN2L* gene variants. The clinical manifestations, electroencephalography, neuroimaging characteristics and treatment of epilepsy were retrospectively analyzed.

Result We identified two new *LMAN2L* compound heterozygous variants, c.476A > G, p.D159G, c.1060_1061del, p.S354Pfs*29, which appeared in two children from the same family. Both cases showed severe postnatal psychomotor developmental lag and developed seizures at 2 months of age, which manifested themselves in a variety of ways and were not relieved by the administration of multiple antiepileptic drugs.

Conclusion Complex heterozygous mutations at the newly identified locus of *LMAN2L* cause refractory epilepsy, with epileptic symptoms beginning at 2 months of age and manifesting as multiple seizure types and developmental delays. This is the first report to link *LMAN2L* to the phenotype of epileptic encephalopathy and refractory epilepsy, suggesting that the heterozygous p.D159G, p.S354Pfs*29 *LMAN2L* variants are likely pathogenic. These 2 newly identified pathogenic variants enrich the spectrum of pathogenic variants in the *LMAN2L* gene.

Keywords Intellectual disability, *LMAN2L*, Epileptic encephalopathy, MRT52

*Correspondence:
Meng Dong
dm850307@126.com
[1]Department of Pediatrics, Qilu Hospital of Shandong University, Jinan, China

Background

Autosomal recessive mental retardation 52 (MRT52, OMIM: 616887) was first reported in 2016, and it is characterized by significantly below average general intellectual functioning associated with impairments in adaptive behavior and manifested during the developmental period [1]. Autosomal recessive mental retardation-52(MRT52) is a subtype of mental retardation whose clinical features include global developmental delay, severe intellectual disability with poor speech, and mild seizures in early childhood. In a large consanguineous Pakistani family, a homozygous missense mutation in the *LMAN2L* gene was found to be segregated from intellectual disability, indicating that this form of mutation in this gene may be a key factor leading to intellectual disability in family members [2]. The studied children had similar clinical phenotypes, all suffering from global developmental delay, severely impaired intellectual development, and poor language ability. These patients also had mild epileptic seizures in early childhood and spontaneously remitted around the age of In addition, studies have shown that dominant *LMAN2L* mutations can cause intellectual disability accompanied by remitting epilepsy. The intellectual disability is manifested as delays and impairments in language, cognitive, and learning abilities. Epileptic seizures mostly occur during childhood, especially during sleep, presenting as generalized tonic-clonic seizures, which can be controlled by drugs and spontaneously remit during puberty. Some patients also have problems with motor coordination disorders, attention deficit hyperactivity disorder (ADHD), as well as behavioral and adaptive functions [3]. This finding further emphasizes the importance of the *LMAN2L* gene in maintaining the function of the nervous system. Its mutations can not only affect intellectual development alone but also are associated with the occurrence of epilepsy. In a Chinese patient with autosomal recessive mental retardation 52 (MRT52), a novel compound heterozygous variant of the lectin mannose binding 2 like gene (*LMAN2L*) was identified, and there was a phenomenon of phenotype expansion [4]. The child in this case also had hearing loss and hypotonia, indicating that different variant types of this gene may all affect intellectual development, and compound heterozygous variants may lead to more complex clinical phenotypes. None of the previously reported cases had malformation features.

Lectin mannose-binding (LMAN) is a family of three ubiquitously expressed proteins (LMAN1, LMAN2, and LMAN2L), which, anchored in the endoplasmic reticulum(ER) through a C-terminal KRFY sequence interact with glucan chains of select glycoproteins to direct them to extracellular secretion [3]. The LMAN2L protein contains an N-terminal signal peptide, an L-type lectin-like carbohydrate recognition domain, a TM domain, and a C-terminal ER retention signal motif [5]. The exact function of *LMAN2L* is not known, but evidence suggests that the *LMAN2L* (OMIM: 609552) encodes for the lectin, mannose-binding 2-like protein which is a cargo receptor in the endoplasmic reticulum important for glycoprotein transport [2, 6].

Here, the present study reports 2 novel loci of *LMAN2L* mutations, expanding the spectrum of *LMAN2L* variants and phenotypes, focusing on seizure and control of epilepsy for clinical diagnosis and treatment.

Methods

Patients

We conducted a retrospective analysis of a family with *LMAN2L* gene mutation admitted to the Department of Pediatrics, Qilu Hospital of Shandong University, where we focused on seizure semiology, EEG abnormalities, psychomotor development, MRI manifestations, and response to treatment. Seizure types were diagnosed and classified according to the guidelines of International League Against Epilepsy (ILAE) (2017 and 2022) [7, 8].

Genetic analyses

DNA extraction from peripheral blood of patients and their parents. Genomic DNA was extracted using a QIAamp Blood Midi Kit (QIAGEN, Valencia, CA). An Illumina Next Seq 500 sequencer (Illumina, San Diego, CA, USA) was used with 150 bp paired-end reads. Clean reads were assembled and spliced using the second-generation sequencing analysis platform provided by MyGenostics and the coverage and sequencing quality of the target region were evaluated. A flash analysis platform was used to analyze the pathogenicity of variation, and possible variation loci were determined. The pathogenicity of variation loci was analyzed according to the ACMG (American College of Medical Genetics and Genomics) genetic variation classification criteria and guidelines [9]. An ABI3730xl sequencer (Applied Biosystems, USA) was used for Sanger sequencing, and the Sanger sequencing results were compared to the capture sequencing results. More details on DNA library preparation, enrichment and sequencing of targeted genes, bioinformatics analysis, the whole genome CNV analysis, variants selected and software and database are listed on the Additional file 1.

Additional file 1: DNA Library Preparation, Enrichment and Sequencing of Targeted Genes, Bioinformatics analysis, The whole genome CNV analysis, Variants Selected, Software and Database.

The *LMAN2L* gene wild-type 3D model was queried using the SWISS-MODEL database (https://swissmodel.expasy.org/). The wild-type model name was A0A5N3W7I3.1.A, covering the range of 1-359 with 61% sequence similarity and 96.10% confidence level. The data

obtained from the homology model were visualized using PyMOL (https://pymol.org/2/).

Result

The mother of the propositus had consulted on assisted reproduction techniques prior to her second pregnancy. Her first child was diagnosed with intellectual disability, had severe developmental delays, was unable to care for herself, and had epileptic seizures at the age of 2 months. Genetic testing was performed after the birth of the child, which revealed mutations at two loci in *LMAN2L*. The proband's parents are healthy and not related by blood. Three generations of collateral blood relatives of the propositus(the mother's cousin, the father's cousin) had epilepsy but no intellectual disability and have not undergone genetic testing. They would like to have genetic counseling to obtain their second child through assisted reproductive technologies. However, the loci of their *LMAN2L* gene mutations have not been certified as pathogenic mutations, which do not meet the indications for third generation in vitro fertilization (IVF). They still conceived their second child (case 2) naturally. After the child was born, he was hospitalized in the neonatal department of our hospital due to feeding difficulties. During this period, genetic testing was completed, and the report showed that there were mutations at the same loci in the *LMAN2L* gene as in case 1 And not long ago, case 2 had convulsive manifestations when he was nearly 2 months old.

Psychomotor development and physical examination

The intellectual disability and psychomotor developmental retardation in Case 1 are more severe than those in the previously reported cases. At the age of 1 year and 3 months, the child still could not hold her head upright; at 11 months, she could not follow objects with her eyes or be amused by others; at the age of 4 years and 11 months, her head holding was still unstable, and she could not sit, crawl or walk, nor could she say "baba, mama". The development levels in multiple aspects such as movement, cognition and language have always remained stagnant. Moreover, this child has limb development deformities that were not mentioned in previous cases. When she was hospitalized for convulsions at the age of 2 months, congenital dislocation of the hip (subluxation of the left hip joint) was found during the physical examination. The child has a low immunity and is hospitalized for severe pneumonia about 4–5 times a year. Physical examination: The body length is above the 97th percentile, and the weight fluctuates between the 15th and 50th percentiles. In the neurological examination, except for persistent low muscle tone, no other abnormalities were found, and the examinations of other systems were normal.

Case 2 was hospitalized in the neonatal unit at birth due tofeeding difficulties (sucking reflex could not be elicited). The neonatal behavioral neurological assessment score was 29 points (out of 40). During hospitalization, bilateral hearing screenings were not passed, and the hearing in both ears is still rather poor at present. Around one week after birth, edema of the lower limbs and perineum with an unknown cause occurred. Auxiliary examinations such as fluid intake and output, albumin, and renal function showed no obvious abnormalities, and then it improved spontaneously. At 1 year and 3 months old, the patient still could not hold the head upright, follow objects with the eyes, or be amused by others. Umbilical hernia was visible on the abdomen during physical examination. Muscle tone was low after birth, and physical examination after the occurrence of convulsions showed high muscle tone in both lower limbs.The body length is between the 50th 85th percentiles, and the body weight is between the 3rd 15th percentiles. (Table 1).

Seizures and EEG features

At nearly 2 months of age, Case 1 had seizure attacks with various forms: generalized tonic-clonic seizures, generalized clonic seizures, myoclonus seizures、focal seizures 、hemiclonic seizures, oral automatisms, and epileptic spasms, including: ① Clenching both hands into fists and making smacking movements, which spontaneously relieved after more than 10 s, and the patient was normal after relief. Afterwards, there were repeated limb tremors, lip - biting and smacking movements, with more

Table 1 Cases clinical features

	case 1	case 2
Sex	Female	Male
Age(months)	47	2
Height(cm)	95	58
Weight(kg)	11	4.35
BW	3.85KG	3.12KG
Gestational age	————	38 + 3 W
Deformities of limb development	Developmental Dysplasia of the Hip	Umbilical hernia
Muscle tone	low	low/Muscle tone is high after seizures
Other systematic examinations	normal	normal

than 30 attacks per day; ②Suddenly shaking the upper or lower limbs once, sometimes with body torsion, which relieved after several seconds, and the attacks occurred frequently every day; ③ Clenching both hands into fists, with limb tremors and smacking movements, lasting for about 1 h, once a month;④ Limb tremors, clenching both hands into fists, and right - forward - looking strabismus of both eyes, once a month. Electroencephalogram (EEG): right lead is characterized by rhythmic interspersed spikes and slow waves of medium-high amplitude 6-7.5cps, ranging from 5 to 16 S each time. Widespread during wakefulness and sleep and multifocal spike waves, polyspike waves, spike - and - slow - waves, slow - spike - and - slow - waves, and polymorphic slow - wave discharges mainly in the right hemisphere, with the right posterior part being prominent; several clonic seizures during the awake period.Sharp - type slow - waves, spike - and - slow - waves, and slow - waves in the anterior and posterior parts of the head; several focal seizures starting from the left occipital, middle and posterior temporal regions. Medium - to - high - amplitude sharp - waves, sharp - and - slow - waves, and polyspike - and - slow - waves were synchronously or asynchronously discharged on both sides. Focal electrical seizures starting from the right occipital - posterior - temporal region. Hypsarrhythmia in the posterior part of the head. (Fig. 1).

At nearly 2 months of age, Case 2 developed tonic clonic seizures, including (i) Toe flexion, tremors in both lower limbs, and right sided strabismus of both eyes, more than 20 times per day; (ii) stiffness of the extremities, tremors in the right foot; (iii) Irregular tremors in all four limbs.; EEG: abnormal infantile electroencephalogram; intermittent burst suppression, with predominantly multifocal spiking, multispiking, spiking-slow, and slow spiking-complex wave issuance in the head after awakening; multifocal initiating focal seizures during awakening and sleep, with 2 wandering focal seizures, frequent wandering myoclonic EEG: intermittent atypical heightened dysrhythmia, posterior head predominant; multifocal spike, slow spike, slow wave issuance in the posterior head; and 4 focal myoclonic seizures in wakefulness. (Fig. 2).

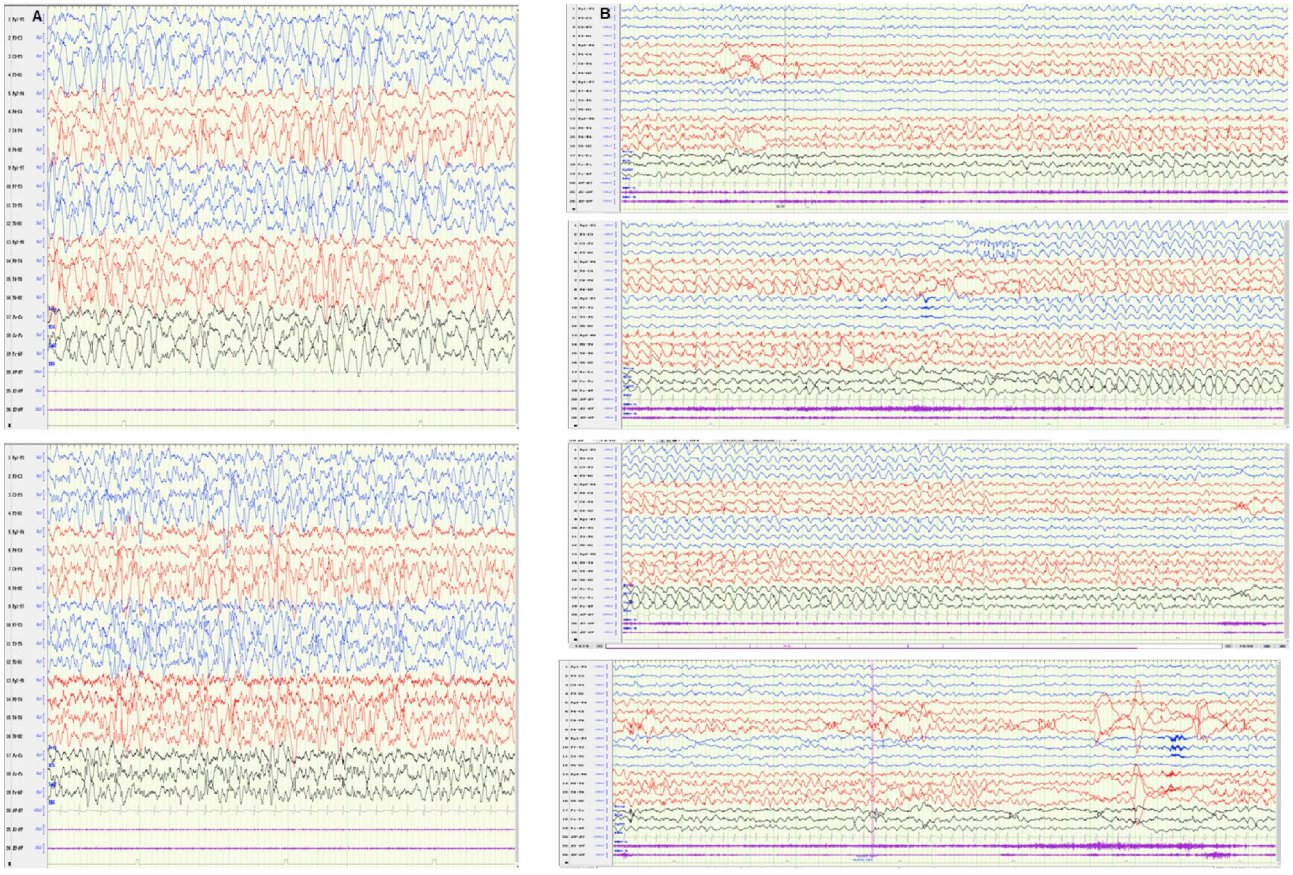

Fig. 1 EEGs in Case 1 with *LMAN2L* gene pathogenic variants; Each green frame is 1 S; X1, X2 left and right deltoids, X3, X4 left and right tibialis anterior muscles; (**A**) hypsarrhythmia; (**B**) Mixed slow-wave discharges of 3–6 Hz with medium-high amplitude starting in the right parietal, occipital, and posterior temporal regions → gradual spreading to the right hemisphere up to full conduction, with persistent widespread medium-high amplitude spiking, spiking, and δ slow-wave discharges

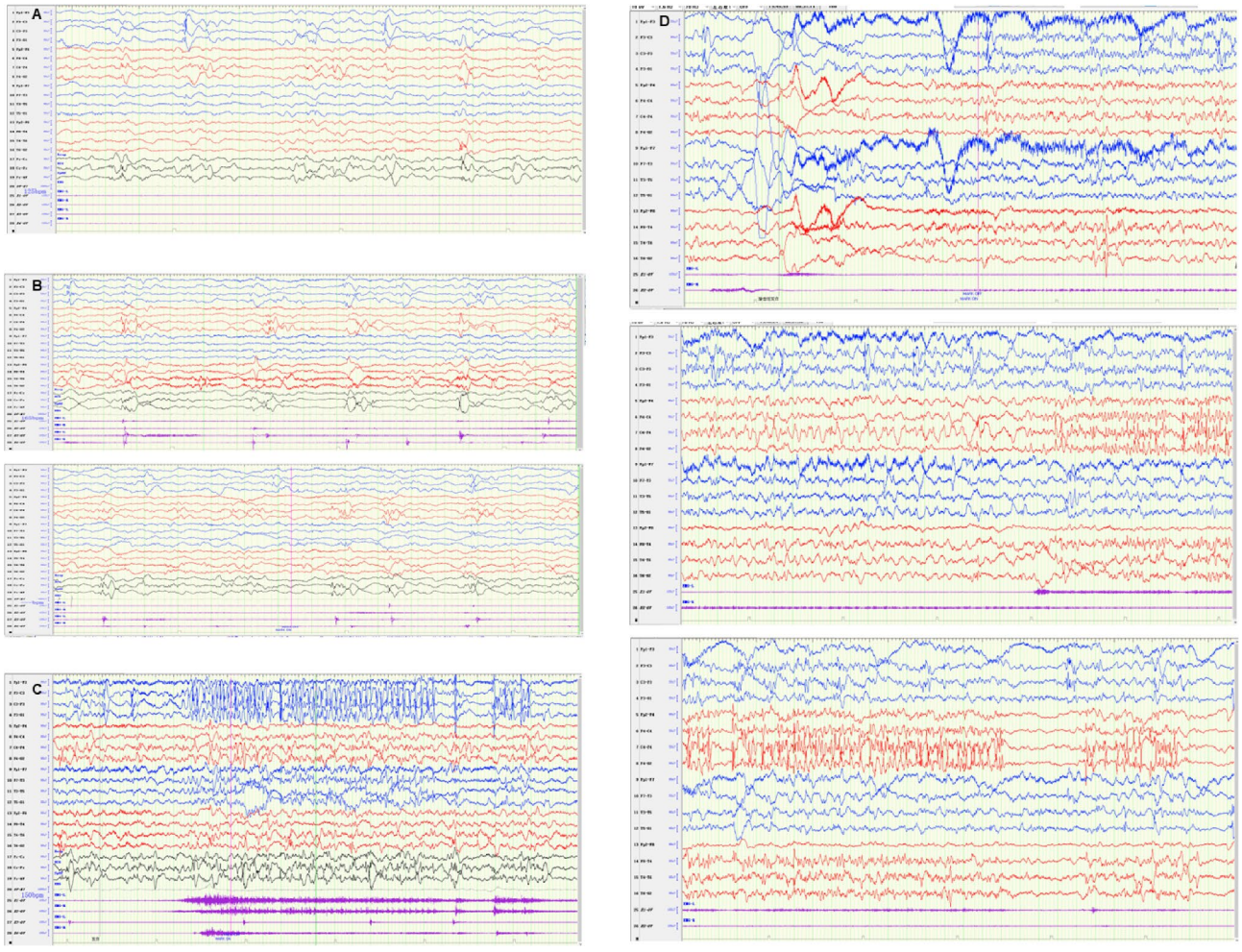

Fig. 2 EEGs in Case 2 with *LMAN2L* gene pathogenic variants; Each green frame is 1 S; X1, X2 left and right deltoids, X3, X4 left and right tibialis anterior muscles; (**A**) Burst-inhibition; (**B**) Wandering focal myoclonic seizures during sleep; (**C**) Focal seizures starting in the left central and parietal regions during wakefulness; (**D**) Focal seizures: wandering from the left central and parietal regions to the right central and parietal regions

Epilepsy response to treatment

In this study, the epilepsy of both children had the characteristic of being difficult to control. For Case 1, drugs such as levetiracetam, topiramate, oxcarbazepine, Depakine (valproate), clonazepam, clobazam, and lamotrigine were given successively. Despite all efforts, epileptic seizures still occurred frequently. High fever could induce status epilepticus in the children. After treatments such as chloral hydrate enema and intramuscular injection of diazepam, it was still difficult to control, and the longest - lasting episode of repeated convulsions was about 11 h. During the most recent follow - up of Case 1, we regretfully found that Case 1 had passed away recently (December 31, 2024, at the age of 4 years and 11 months). The death diagnosis was "severe pneumonia, septicemia with shock, respiratory failure, multiple - organ failure, status epilepticus, and metabolic acidosis". During the course of the disease, the child had status epilepticus induced by fever again, and it was still difficult to relieve even with continuous infusion of midazolam.

After "Depakine, Keppra, and Topamax" were given to Case 2, the epileptic seizures were still not well - controlled. ACTH therapy was given at the age of 5 months. During the shock process, it could be observed that the child had fewer seizures than before. Unfortunately, during the long - term follow - up of this child, the child had frequent epileptic seizures again. In the course of Case 1, hypsarrhythmia once occurred (Fig. 1A). The manifestations of convulsions were: clenching both hands into fists, and the four limbs becoming stiff and lifting up. We once planned to perform ACTH therapy on Case 1, but the family members refused.

Ancillary examinations

The first-line examinations (infection screening and metabolic screening) for epileptic encephalopathy are normal, including no abnormalities found in serum lactic

Table 2 :Laboratory results

	Case1	Case2(Convulsions before)	Case2(after convulsions)
LDH U/L	387	540	377
CK U/L	209	336	142
CKMB ug/ml	0.6	16.8	9.1
Blood biochemistry	normal	normal	normal
Liver function, kidney function	normal	normal	normal
Lacticacid mmol/L	1.9	1.6	3.6
blood ammonia umol/L	33→71→28→137		23
hcy umol/L	4.5		5.6
ESR mm/H	4		2
Ceruloplasmin mg/L	204.5		

[i]CKMB, creatine kinase isoenzymes; CK, Creatine Kinase; hcy, homocysteine; ESR, Erythrocyte Sedimentation Rate

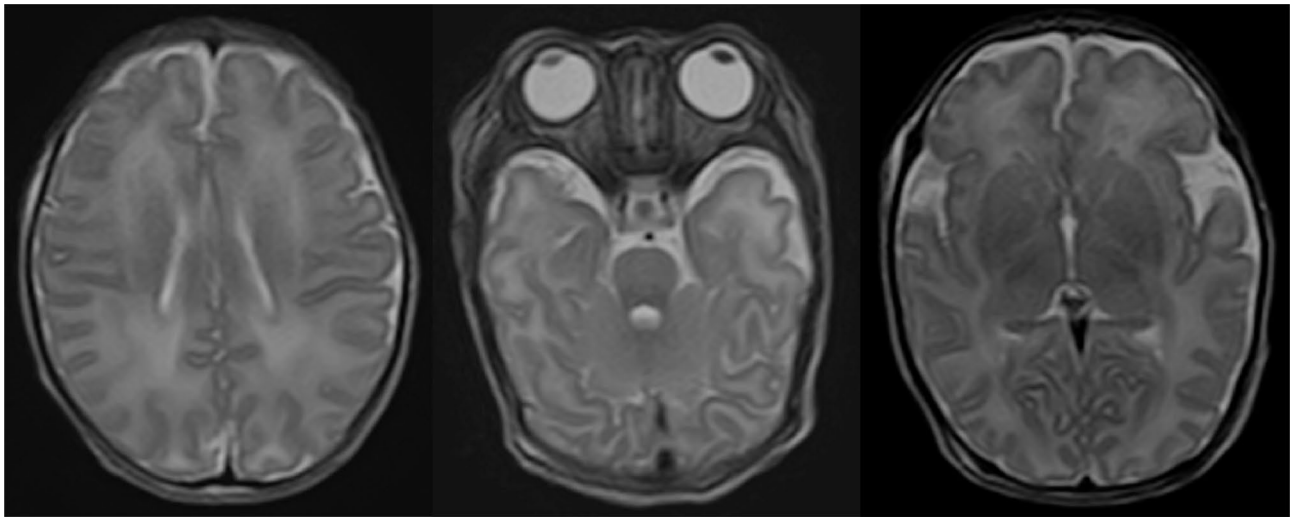

Fig. 3 The MRI of Case 2

acid, blood ammonia, erythrocyte sedimentation rate, and ceruloplasmin. (Table 2).

MRI

The patient's MRI was performed to look for abnormalities visible on structural neuroimaging to determine if the epileptic symptoms were caused by a structural etiology [7]. No significant abnormalities were seen on MRI in Case 1 or 2. Previous studies have shown that patients usually have no or little cognitive impairment or neurodevelopmental comorbidity if the neuroimaging findings are normal [10]. However, in our clinical study, Case 1 and 2 had normal MRI findings, but the children showed varying degrees of language and behavioral deficits. (Fig. 3)

Detection and genetic analysis of pathogenic variants in the *LMAN2L* gene

In Case 1 and 2, the *LMAN2L* gene (NM_001142292.2) has two potentially pathogenic variants in a heterozygous state. The c.476A>G variant is located in exon 4, and the c.1060_1061del variant is in exon 9. The c.476A>G (p.D159G) mutation is inherited from a phenotypically normal mother. This c.476A>G mutation leads to an amino acid substitution (p.D159G). Through the structural analysis of the LMAN2L protein, it was found that the identified c.476A>G (p.D159G) mutation changes amino acid 159 from aspartic acid to glycine. Before the mutation, it forms four hydrogen bonds with threonine at position 160, tryptophan at position 161, and tyrosine at position 185. After the mutation, it forms only two hydrogen bonds with tyrosine at position 185, with a reduction of two in the number of hydrogen bonds. This may have an impact on the protein conformation.According to the criteria of the American College of Medical Genetics and Genomics (ACMG), the c.476A>G (p.D159G) variant was initially classified as VUS (variant of uncertain significance) with PM2 + PP3. Bioinformatics protein function prediction software such as SIFT, PolyPhen-2, MutationTaster, GERP++, and REVEL all predicted that these mutations were deleterious. This variant is located in the carbohydrate recognition domain (as shown in Fig. 4) [4]. Thus, this variant may change the sugar-binding affinity and impair the function.

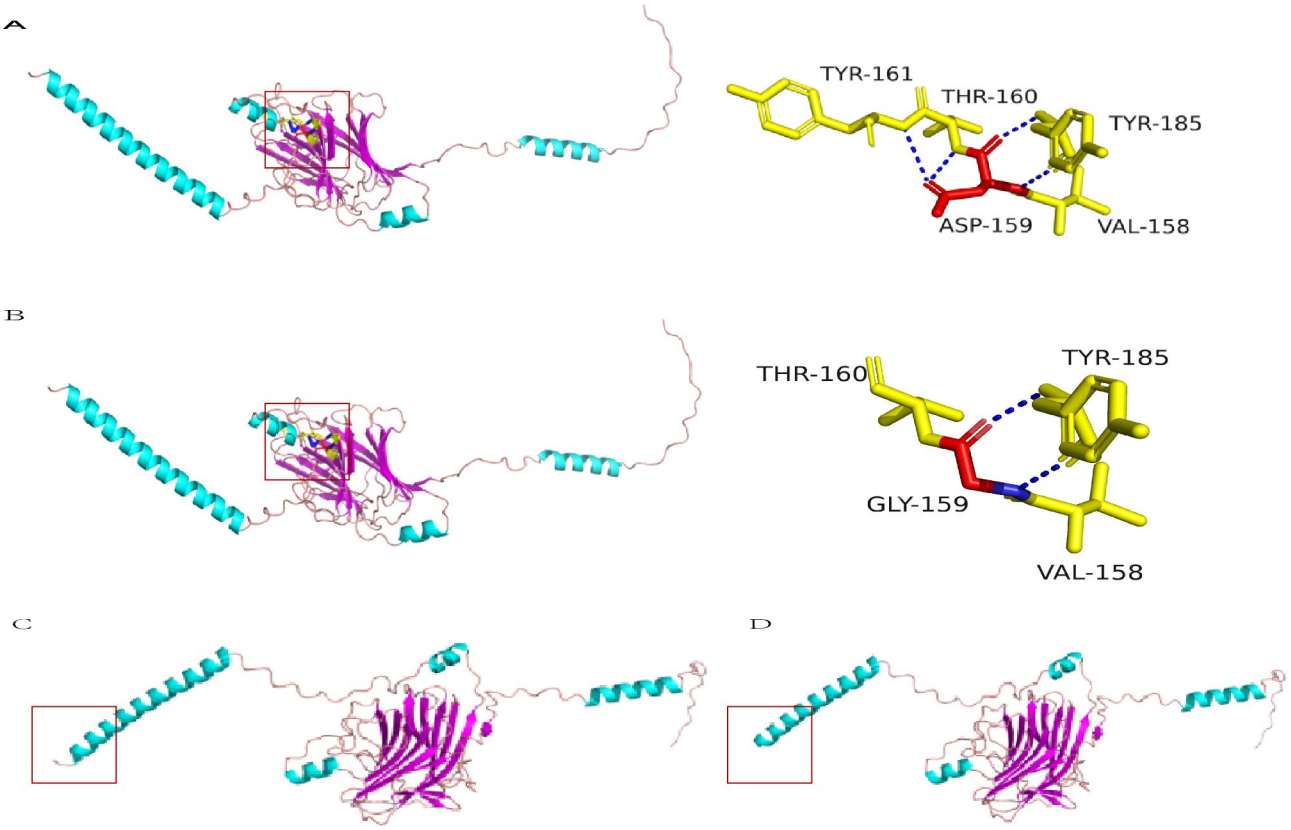

Fig. 4 *LMAN2L* three-dimensional structure model. The mutation positions are highlighted by boxes; in the cartoon structure, blue represents the α-helix, purple represents β-folding, and pink coils represent the loop structure. Figure shows the hydrogen bonding observed as a stick structure, where each color represents a different atom, yellow-C atoms, gray-H atoms, blue-N atoms, red-O atoms, or orange-S atoms, and the green dashed line is the hydrogen bond. (**A**) In the *LMAN2L* wild type, amino acid 159 is aspartic acid and forms 4 hydrogen bonds with threonine at position 160, tryptophan at position 161, and tyrosine at position 185; (**B**)in the *LMAN2L* c.476A>G mutant, amino acid 159 is glycine, and forms 2 hydrogen bonds with tyrosine at position 185, and the number of hydrogen bonds is reduced by 2 compared to that in the wild type, which may affect protein conformation; (**C**, **D**) in the human *LMAN2L* protein predicted changes caused by mutation the p.S354Pfs*29 mutation, amino acid 354 is changed from serine to proline, and the movement of 27 amino acids to encounter a stop codon, may affect protein conformation

A mutation at the c.1060_1061del (p.S354Pfs*29) locus was inherited from a phenotypically normal father. The mutation at this site changes amino acid 354 from serine to proline, moving 27 amino acids to encounter a termination codon that may affect protein conformation.

Discussion

Consistent with previous reports, our study subjects presented with global developmental delay, severe mental retardation, speech impairment, and seizures [1, 3, 4]. Case 2 had Hearing impairment at birth, and Cases 1 and 2 had hypotonia, which were consistent with the findings reported by Cong Zhou et al. (2023) [4]. None of the previously reported cases were characterized by malformations. In this report, Case 1 had developmental dysplasia of the hip while Case 2 presented with an umbilical hernia and failed bilateral hearing screening at birth. We have followed up with him, and as of this date, his binaural hearing is still unsatisfactory, which is consistent with that reported by Cong Zhou et al. (2023) [4]; Cases 1 and 2 also had hypotonia, with the difference that Case 2's hypotonia shifted from low to high after the onset of the seizure, and did not change again. What is different about the cases in this study compared to previous reports is that there were also recurrent respiratory tract infections. They were hospitalized for pneumonia many times each year. Case 1 passed away when nearly five years old during hospitalization for "respiratory tract infection".

Compared with the previously reported cases, the two cases in this study had more severe, more frequent, more difficult-to-control seizures and were more prone to persistent status epilepticus. In the cases reported by Rafiullah R et al. (2016), five patients had mild seizures before the age of 5, and the seizures began in the first year of life and stopped spontaneously without any medication.In the cases reported by Reem A et al. (2019), the seizures were mild and all seizures disappeared after the patients entered puberty.In the cases reported by Cong Zhou et al. (2023), the patient had a sudden seizure at the age of 2 months and was treated with keplan and levetiracetam

for epilepsy with poor efficacy, which is consistent with our study. The case reported by Cong Zhou et al. (2023) had a compound heterozygous variant of *LMAN2L*, so we considered whether the compound heterozygous mutation of *LMAN2L* could lead to more severe seizures.

Epileptic encephalopathy refers to the fact that frequent epileptiform-like activities have an adverse impact on development, usually resulting in a slowdown or regression of developmental skills and are generally associated with frequent epileptic seizures. When the encephalopathy state in children with epilepsy is caused by the dual effects of underlying developmental abnormal etiologies and epilepsy, it is called developmental epileptic encephalopathy. Most of these disorders occur in the neonatal, infant or childhood periods, with significantly abnormal electroencephalograms and poor drug treatment effects. We believe that the frequent and difficult-to-control epileptic seizures in Case 1 and Case 2 have further aggravated the degree of their intellectual disabilities. However, the physical development of the children has not shown obvious retardation, and the heights of Case 1 and Case 2 are both above the 50th percentile of their age groups. In the report by Cong Zhou (2023), it was speculated that the hearing impairment of the child led to a more severe degree of intellectual disability than in previously reported cases, and this study also seems to confirm this point. The degree of intellectual disability in Case 2 with hearing impairment is more severe than in previously reported cases.

Case 1 and Case 2 have been successively given a variety of antiepileptic drugs. We also tried ACTH therapy on Case 2, but the epileptic seizures are still difficult to control. For some patients with drug-resistant epilepsy, the ketogenic diet has been proven to have a certain curative effect [11, 12]. The ketogenic diet requires strict diet management and may have the problem of poor compliance for most children with epilepsy. However, since the two children in this study are unable to take care of themselves, the implementation of the ketogenic diet may be more easily achieved. But there are multiple risks in the practice of the ketogenic diet. Therefore, if the ketogenic diet is to be used to treat the patients in this case, a multidisciplinary team (including neurologists, dietitians, etc.) needs to cooperate to develop a personalized diet plan and continuously evaluate the treatment effect and safety. Steroid therapy is also a potential alternative therapy that has attracted much attention. Judging from the results of the multicenter study in Falsaperla R et al. (2024), steroid therapy has achieved a certain degree of curative effect in some patients with drug-resistant epilepsy [13]. Especially in epilepsy related to autoimmune mechanisms, steroid therapy has achieved good clinical results, and some patients with drug-resistant epilepsy caused by genetic factors also respond to steroid treatment which suggests to us that for the two children mentioned in this study, steroid therapy may also have potential therapeutic effects. However, the two children in this study have poor physical fitness, extremely backward development, and are bedridden for a long time. Using steroid therapy may increase their risk of infection. Therefore, it is necessary to comprehensively consider the specific situation of the children and weigh the treatment benefits and risks. However, as potential alternative therapies, the ketogenic diet and steroid therapy have certain exploration value in the treatment of this case. In the future, we look forward to further research and clinical practice to deeply understand the efficacy and safety of these therapies in refractory epilepsy related to the *LMAN2L* gene and provide more effective treatment options for patients.

The mutation at the c.476A>G (p.D159G) site was inherited from a mother with a normal phenotype, and the mutation at the c.1060_1061del (p.S354Pfs*29) site was inherited from a father with a normal phenotype. In combination with the results of whole - exome sequencing (WES), we identified two *LMAN2L* compound heterozygous variants in Case 1 and Case 2, and both of them were heterozygous carriers of this gene. Therefore, these findings indicate an autosomal recessive inheritance pattern, similar to that reported in Cong Zhou et al. (2023) and the family from Pakistan et al. (2016). Case 1 and Case 2 had the same variation loci and similar clinical phenotypes. Sanger sequencing confirmed the existence of both variants in the previous samples. Analysis of parental DNA showed that they were heterozygous carriers. (See Figs. 5 and 6)

LMAN2L encodes a protein that belongs to the L-type lectin group of type 1 membrane proteins and can bind to glycoproteins to assist their transport to the Golgi apparatus [14]. *LMAN2L* may contribute to the receptor-mediated transport of mature glycoproteins out of the endoplasmic reticulum (ER) in COPII-coated vesicles [15]. The mutation at the c.476A>G (p.D159G) site changes amino acid 159 from aspartic acid to glycine. Before the mutation, aspartic acid forms four hydrogen bonds with threonine at position 160, tryptophan at position 161, and tyrosine at position 185. After the mutation, it only forms two hydrogen bonds with tyrosine at position 185. The reduction in the number of hydrogen bonds is likely to cause a change in the local conformation of the protein, and this variant site is located in the carbohydrate-recognition domain, which may change the glycan-binding affinity and disrupt glycoprotein transport [4]. The mutation at the c.1060_1061del (p.S354Pfs*29) site changes amino acid 354 from serine to proline and encounters a stop codon after moving 27 amino acids, resulting in premature termination of protein synthesis and the formation of a truncated protein. The new amino acid sequence arrangement and the

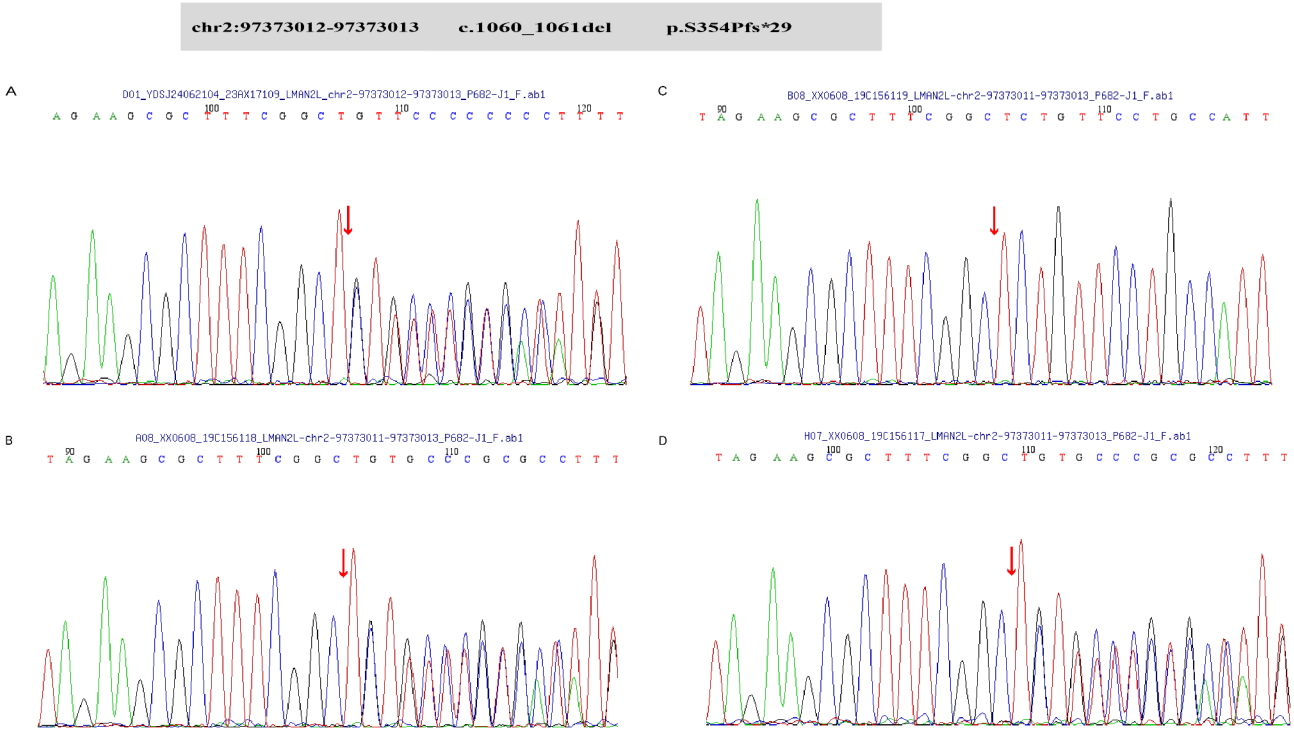

Fig. 5 Sanger sequencing shows novel *LMAN2L* compound heterozygous variant inherited from the parents. c.1060_1061del (p.S354Pfs*29) variant

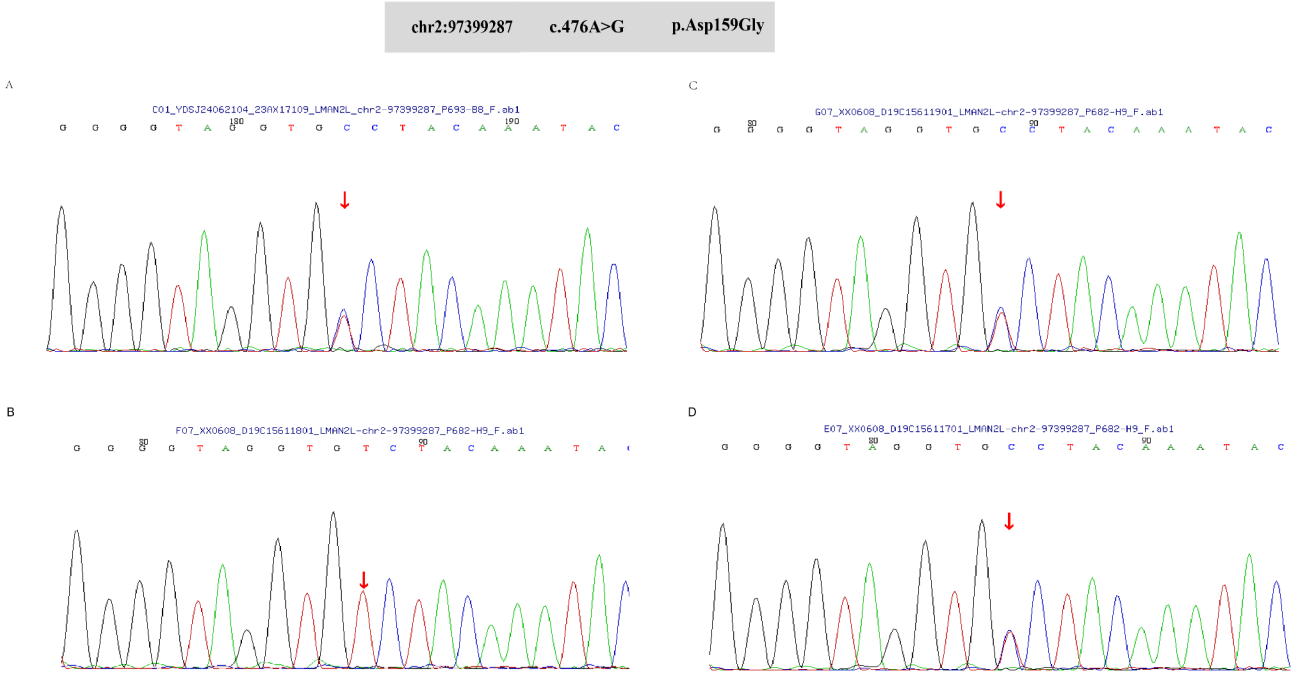

Fig. 6 Sanger sequencing shows novel *LMAN2L* compound heterozygous variant inherited from the parents. c.476A>G (p.D159G) variant

prematurely occurring stop codon may lead to abnormal protein folding and generate an incorrect conformation. This abnormally-conformed protein may not be able to function normally in glycoprotein transport. When the *LMAN2L* gene is mutated, misfolded glycoproteins may accumulate intracellularly, which may trigger endoplasmic reticulum stress, and the accumulation of misfolded glycoproteins and endoplasmic reticulum stress may stimulate a neuroinflammatory response. These situations may affect the normal function of neurons and may

thus lead to the occurrence of epilepsy [16]. However, more in- depth research in the future is still required to further clarify the details of the molecular mechanisms at each step, in the hope of finding intervention targets and bringing new breakthroughs in the treatment of epilepsy.

Conclusions

Complex heterozygous mutations at the newly identified locus of *LMAN2L* cause refractory epilepsy, with epileptic symptoms beginning at 2 months of age and manifesting as multiple seizure types and developmental delays. This is the first report to link *LMAN2L* to the phenotype of epileptic encephalopathy and refractory epilepsy, suggesting that the heterozygous p.D159G, p.S354Pfs*29 *LMAN2L* variants are likely pathogenic. These 2 newly identified pathogenic variants enrich the spectrum of pathogenic variants in the *LMAN2L* gene.

In conclusion, we report two cases of patients with compound heterozygous variants of the *LMAN2L* gene and atypical presentations, expanding the recognized phenotype, with new variants extending the *LMAN2L* spectrum.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13052-025-01960-6.

Supplementary Material 1

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Author contributions

TW and MD conceived this research. TW 、YG、PY and YhY collated clinical information, and collected behavioral phenotyping data. MD carried out the genetic analysis. LLT underwent an EEG analysis. MD made critical revisions to the important intellectual content of the manuscript. All authors wrote, revised, and approved the manuscript.

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Data availability

The datasets generated and/or analysed during the current study are not publicly available due to government restrictions but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Qilu Hospital, Shandong University, Ethics Approval No. KYLL-202407-029-1.All participants or their guardians provided informed consent and signed the informed consent form.

Consent for publication

We obtained Informed consent of the patients' parents to publish this article.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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