BMC Medical Genomics

CASE REPORT

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A novel variant of biallelic *MME* gene associated with autosomal recessive late-onset distal hereditary motor neuropathy in Chinese families

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Abstract

Distal hereditary motor neuropathies (dHMN) are a group of heterogeneous diseases and previous studies have reported that the compound heterozygous recessive *MME* variants cause dHMN. Our study found a novel homozygous *MME* variant and a reported compound heterozygous *MME* variant in two Chinese families, respectively. Next-generation sequencing and nerve conduction studies were performed for two probands. The probands in two families presented with the muscle weakness and wasting of both lower limbs and carried a c.2122 A>T (p.K708*) and c.1342 C>T&c.2071_2072delinsTT (p.R448*&p.A691L) variant, respectively. Prominently axonal impairment of motor nerves and slight involvement of sensory nerves were observed in nerve conduction study. Our study reported a "novel" nonsense mutation and a missense variant of autosomal recessive late-onset dHMN and reviewed reported *MME* variants associated with dHMN phenotype.

Keywords Distal hereditary motor neuropathies, *MME*, CMT2, Late-onset, Peptidase M13 domain

Introduction

Distal hereditary motor neuropathies (dHMN) comprise a group of clinically and genetically heterogeneous diseases characterized by distal lower motor neuron weakness without severe sensory involvement [1]. The typical dHMN phenotype exhibits slowly progressive weakness and atrophy of distal lower limb muscles starting in either childhood or adulthood [2]. Many gene mutations, such as *alanyl-tRNA synthetase 1* (*AARS1*), *bicaudal D homolog 2* (*BICD2*), *Berardinelli-Seip congenital lipodystrophy 2* (*BSCL2*), *membrane metallo-endopeptidase (MME)*, *sorbitol dehydrogenase (SORD)*, *and synaptotagmin II (SYT2)* [2], are associated with dHMN. Despite many gene mutations that have been confirmed in dHMN,

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there are more than half of patients still without exact genetic causes [3].

MME encodes membrane metallo-endopeptidase, which is a zinc-dependent metalloprotease and also known as neprilysin or neutral endopeptidase (NEP) that cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones [4]. Neprilysin exists predominantly as a homodimer on the cell surface in many tissues and plays important roles in kidney proteolytic processes, cardiovascular regulation, immune response and cell proliferation [5, 6]. It is a β-amyloid (Aβ) degrading enzyme which makes it a potential therapeutic target in Alzheimer's disease (AD) [6]. Besides, *MME* mutations have been associated with peripheral neuropathy, such as axonal Charcot-Marie-Tooth neuropathy (CMT2) [7], spinocerebellar ataxia with neuropathy (SCA43) [8] and dHMN [9]. Many forms of dHMN have minor or subclinical sensory involvement and overlap with CMT2, as the same gene mutation may cause both phenotypes [10]. Previous studies have identified the association between autosomal dominant or recessive *MME* mutations and CMT2 [11]. In this study, we introduced two dHMN families with *MME* variants and reviewed clinical and genetic features of reported *MME* gene-related dHMN.

Methods

Subjects

Two nonconsanguineous families with *MME* variants were included in this study. Clinical evaluations were conducted and manifestations were collected in affected family members by experienced neurologists. The electromyogram and next-generation sequencing (NGS) were performed on two probands. All individuals involved in this study signed the written informed consent before they were enrolled in the study.

Nerve conduction study

Detection method of nerve conduction in our hospital: CKP electromyograph of Vidi Company was used to measure nerve conduction velocity. The patient lies supine, with the indoor temperature of 20℃ ~ 22℃ and the skin temperature of 32℃ ~ 34℃. (1) Sensory nerve conduction examination: needle electrode was used for recording electrode and reference electrode, saddle electrode was used for stimulating electrode, bilateral median nerve, ulnar nerve and sural nerve were stimulated by forward method, and the stimulation intensity was less than 20mV, the frequency was 1 Hz, the stimulation duration was 0.2ms, the bandpass was 20 ~ 3000 Hz, and the sensitivity was 20μV/cm. Sensory conduction velocity (SCV) and sensory nerve action potential (SNAP) were recorded. (2) Motor nerve conduction examination: the recording electrode and the reference electrode were surface electrodes, and the stimulation electrode was saddle electrode, with the stimulation duration pulse width of 0.2ms, the stimulation frequency of 1 Hz, the bandpass of 2 ~ 10 kHz, the sensitivity of 5mV/cm and the analysis room of 50 ms. The bilateral median nerve, ulnar nerve, tibial nerve and common peroneal nerve were detected, and the distal motor latency (DML), motor conduction velocity (MCV) and compound muscle action potential (CMAP) of motor nerve conduction were recorded.

Next-generation sequencing (NGS)

Genomic DNA was extracted from peripheral blood samples from the probands and their family members using the method of salt precipitation. The genetic test was performed in the proband using targeted NGS that involved Agilent SureDesign Panel Kit (0.4 Mb, for neuromuscular disorders) (Agilent, Santa Clara, CA, USA) and the Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA, USA). Sanger sequencing with specific primers was done to confirm the variants. Further analysis of genetic variants was done among other family members.

Results

Clinical manifestations

The first proband was a 63-year-old woman from a non-consanguineous family A (Fig. 1A II:1). She initially had muscle weakness in both lower limbs at age 58. She walked slowly on crutches at first and then her hands were not as flexible as before. Physical examination revealed that she presented with an abnormal gait and muscle atrophy in her feet and hands (Fig. 1C). Sensory disturbances were not detected in the limbs. Her muscle strength grade was 3+/5 in both lower proximal limbs, 2/5 in both lower distal limbs, and 4/5 in both upper limbs. Deep tendon reflexes were normal in both lower limbs. The Babinski's sign was positive on the right side. The clinical manifestations are summarized in Table 1.

The second proband was a 56-year-old woman with difficulties in squatting and walking on her toes (Fig. 1B II:1). She first complained of walking upstairs with a handrail and a slow walk on uneven ground at the age of 51. And she had a steppage gait at 54, and could not walk on her toes at 55. Her right wrist was fractured from the fall and strength diminished. The strength grades of both upper limbs were 5/5, both lower proximal limbs were 5-/5, and the lower distal limbs were 3/5. Physical examination revealed the atrophy of bilateral gastrocnemius and tibialis anterior muscle. Pain, light touch, vibration, and joint position sensations were intact. Achilles tendon reflexes were not elicited on both sides. The Babinski's sign was negative on both sides.

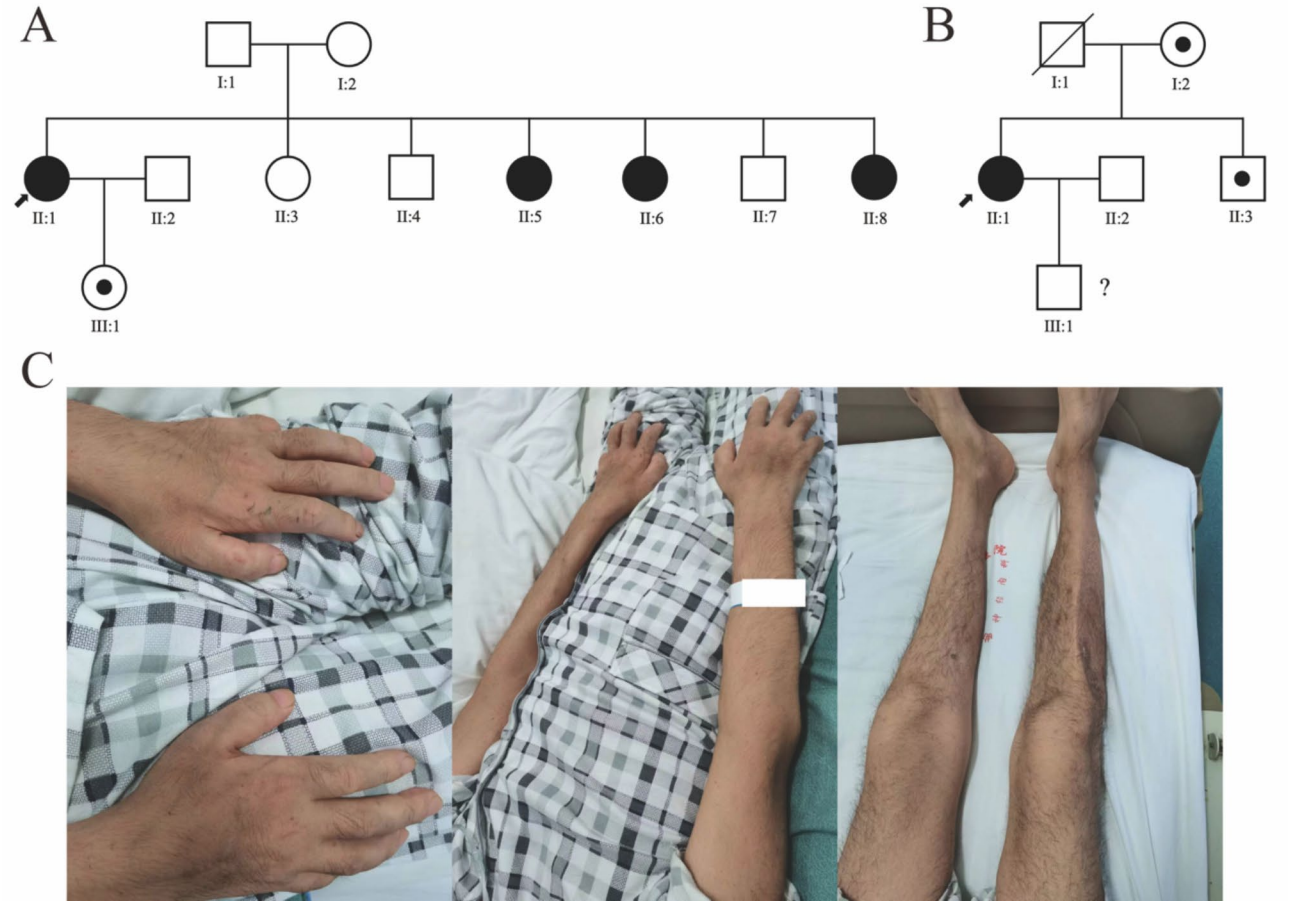

Fig. 1 Family pedigrees and clinical manifestations of patients with *MME* variants. (**A**) Family pedigree A shows five patients (arrow indicates the proband II:1, black square indicates the other patient, + indicates mutant allele, - indicates wild-type allele). (**B**) Family pedigree B shows patient II:1 with other MME mutation (- indicates another mutant allele). (**C**) Patient II:1 of family A

Nerve conduction study

The nerve conduction studies were performed on two probands. For patient A II:1, all sensory nerve conduction velocities (SNCV) were slightly to moderately decreased, while the amplitudes of sensory nerve action potential (SNAP) were normal. The amplitudes of compound motor action potential (CMAP) were significantly decreased with mildly slow motor nerve conduction velocities (MNCV) and almost normal distal motor latency (dL) for bilateral median and ulnar nerves. The CMAP was not recorded for bilateral tibial and peroneal nerves.

For patient B II:1, the SNCV of right median nerve and bilateral ulnar nerves were mildly decreased, and the amplitudes of SNAP were normal for all detected sensory nerves except left sural nerve. The amplitudes of CMAP were significantly decreased for left tibial and bilateral peroneal nerves. The MNCV and dL were normal for all detected nerves except right peroneal nerve and left tibial nerve with slightly decreased MNCV and moderated increased dL. The parameters of motor nerve conduction were not recorded for right tibial nerve (Table 2).

Genetic analysis

NGS revealed a homozygous nonsense variant and a compound heterozygous variant in two families, respectively. For family A II:1, 5, 6, 8, we identified the same homozygous mutation in the splice site c.2122 A>T in exon 22 of *MME* gene, resulting in amino acid of Lysine to a termination codon p.K708* in the Peptidase M13 domain of the protein (Fig. 2D). The variant was neither found in Exome Aggregation Consortium nor the 1000 Genomes. The K708* was highly conserved among different species (Fig. 2C). In silico prediction software, Mutation Taster (http://www.mutationtaster.org/) as disease-causing (Score of 1.0). Mutation nomenclature was based on RefSeq NM_007289.4 (Genbank) for *MME* cDNA, and followed the guidelines of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). According to the guidelines and standards of American College of Medical Genetics and Genomics (ACMG), the variant was classified as pathogenic. The homozygous variant was also identified in her three sisters who had similar symptoms (Fig. 2A). We did not detect pathogenic variants in other genes associated with

Table 1 The clinical features of two probands in *MME* gene related dHMN

variable	Family A II:1	Family B II:1
Gender	Female	Female
Family history	+	-
Age at onset	58	51
Age at examination	63	56
Symptoms at onset	Weakness of LL	Weakness of LL
Abnormal gait	Steppage gait	Steppage gait
Muscle wasting		
Upper limbs	-	-
Lower limbs	+	+
Muscle weakness		
Upper limbs		
proximal limbs	4	5
distal limbs	4	5
Lower limbs		
proximal limbs	3+	5-
distal limbs	2	3
Babinski reflexes	+	-
Knee reflexes	+	+
Ankle reflexes	+	-
Sensory symptoms	-	-
Mutation		
Nucleotide	c.2122 A > T	c.1342 C > T&c.2071_2072delinsTT
Amino acid	p.K708*	p.R448*&p.A691L
Electrophysiological examination	Motor neuropathy with mild sensory nerve involvement	Motor neuropathy

Table 2 Nerve conduction studies of the patients with *MME* mutations

	Family A II:1			**Family B II:1**			**Normal values**		
Motor nerve	MNCV (m/s)	dL (ms)	CMAP (mV)	MNCV (m/s)	dL (ms)	CMAP (mV)	MNCV (m/s)	dL (ms)	CMAP (mV)
L Median	**43.5**	**4.1**	**2.5**	52.5	3.08	9.1	> 50	< 4	> 5
R Median	**43.7**	**4.1**	**3.7**	58.3	3.9	5.2			
L Ulnar	51.2	2.8	**2.8**	51.7	2.85	5.0	> 50	< 3	> 4
R Ulnar	**42.3**	3.0	**1.8**	55.2	2.98	6.1			
L Peroneal	**NR**	**NR**	**NR**	43.5	4.27	**1.26**	> 40	< 5.3	> 2
R Peroneal	**NR**	**NR**	**NR**	**38.6**	**7.2**	**0.22**			
L Tibial	**NR**	**NR**	**NR**	**36.7**	**6.17**	**0.21**	> 40	< 5	> 3.5
R Tibial	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**			
Sensory nerve	SNCV (m/s)		SNAP (μV)	SNCV (m/s)		SNAP (μV)	SNCV (m/s)		SNAP (μV)
L Median	**38.5**		38.5	55.4		7.0	> 50		> 5
R Median	**36.1**		49.9	**47.3**		16.8			
L Ulnar	**49.3**		95.3	**46.0**		14.5	> 50		> 3
R Ulnar	**40.0**		84.3	**48.3**		25.3			
L Sural	**25.0**		41.5	57.1		**0.45**	> 40		> 1
R Sural	**20.5**		13.6	61.0		6.3			

Abbreviations: CMAP, compound motor action potential; dL, distal motor latency; MNCV, motor nerve conduction velocity; NR, not recorded; SNAP, sensory nerve action potential; SNCV, sensory nerve conduction velocity;

All abnormal values are printed in bold

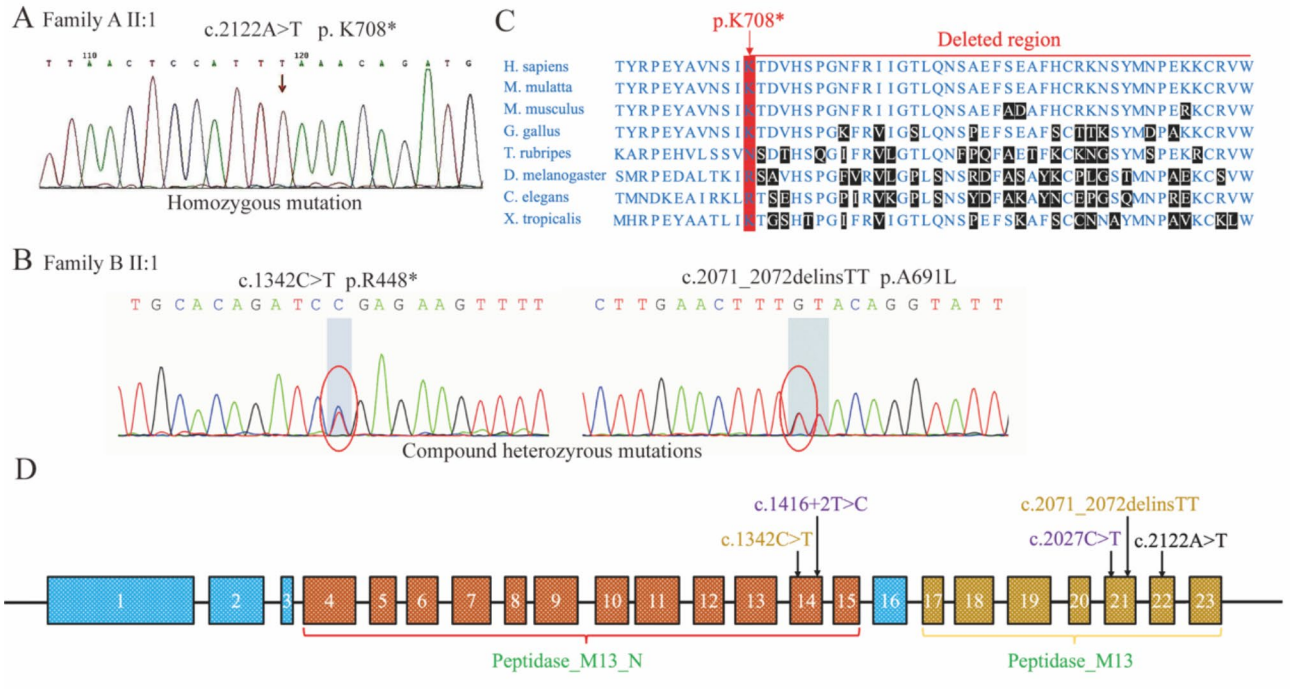

Fig. 2 The genetic variants of dHMN patients in *MME* mutation. (**A**) and (**B**) The Electrophoregrams of two dHMN probands. (**C**) The high evolutionary conservation of residue lysin 708. (**D**) Schematic representation of *MME* gene (NM_007289.4) and reported dMHN variants

inherited peripheral neuropathies in family A. For family B II:1, the compound heterozygous mutation c.1342 C>T&c.2071_2072delinsTT (p.R448*&p.A691L) was identified in proband, and had been reported as pathogenic variants (Fig. 2B) [9]. Her father had died, but the genetic analysis showed her mother and brother each carrying one heterozygous *MME* variant. Therefore, the proband B II:1 carried a compound heterozygous *MME* variant of dHMN.

Discussion

Distal hereditary motor neuropathies (dHMN) are a clinically and genetically diverse range of disorders characterized by progressive degeneration of the distal lower motor neuron without significant sensory involvement. It was originally classified into seven subtypes based on the mode of inheritance and phenotype and has been expanded by some additional types [12]. Some dHMN patients with *HSPB1* gene mutations were reported to have reduced SNAP amplitudes [13], indicating that dHMN may have minor or subclinical sensory involvement. Several forms of dHMN overlap with CMT2 and some gene mutations can cause both phenotypes [1]. Furthermore, there exists a continuum between CMT2 and dHMN forms [14], and some dHMN patients may present with sensory symptoms and close to the diagnosis of CMT2 as the disease progresses. Here, we introduced two late-onset consanguineous families with biallelic *MME* variants suffering from dHMN. Both probands presented with the same clinical features including late-onset muscle weakness and atrophy of lower limbs, walking difficulty and abnormal gait, without sensory symptoms or signs. The nerve condition study showed the motor nerves of lower limbs were severely affected. Although the amplitudes of SNAP and SNCV were mildly abnormal, patients did not present with the sensory symptoms. There is little evidence for sensory involvement is not enough evidence to indicate sensory involvement and hence the phenotype is properly described as dHMN.

The *MME* mutations have been reported associated with autosomal recessive dHMN [9]. There were two *MME* variants, c.1342 C>T&c.2071_2072delinsTT and c.1416+2T>C&c.2027 C>T identified by Daojun Hong et al. In reported *MME* variants, all patients presented with progressive lower limb weakness, abnormal gait and muscle wasting, and some showed mild sensory involvement and upper limb weakness. Patient with c.1342 C>T&c.2071_2072delinsTT variant was late-onset while c.1416+2T>C&c.2027 C>T variant was juvenile-onset. In our study, both *MME* variants caused late-onset dHMN. Therefore, the *MME* mutations might cause both juvenile-onset and late-onset. Besides, the same variant (c.1416+2T>C&c.2027 C>T) was reported in our study and the electromyogram showed the reduced SNAP amplitude of left sural nerve compared with the no sensory abnormality reported by Daojun Hong et al. [9], indicating that sensory involvement existed and

could appear in autosomal recessive variants. Although *MME* variants associated with dHMN were identified, there were more *MME* variants reported causing CMT2. Previous studies had identified *MME* variants in autosomal recessive CMT2, and all patients showed muscle weakness, atrophy, and sensory disturbance in the lower extremities [7]. Interestingly, some of *MME* mutations related to both dHMN and CMT2, and dHMN and CMT2 might belong to a continuum of *MME*-related disorders [9]. Furthermore, Babinski's sign was positive in family A II:1, indicating pyramidal involvement in MME mutations. Previous studies have reported some *MME*-related amyotrophic lateral sclerosis (ALS) and patients had bulbar signs and brisk patellar tendon reflex [10]. Besides *MME*, many gene mutations such as KIF5A [15], DCTN1 [16] and VRK1 [17], could manifest as dHMN and ALS respectively. Therefore, the *MME*-related disorders are more extensive and ALS may be included in it. For histopathology, previous sural nerve biopsy from dHMN patients showed a relatively normal density and structure of nerve fibers and expression of *MME* mildly decreased [9], while densities of large myelinated fibers from CMT2 were markedly decreased and *MME* expression decreased [7], suggesting the histopathological heterogeneity in *MME*-related disorders.

MME protein is the prototype of a group of metallopeptidases belonging to the M13 subfamily of mammalian neutral endopeptidases and consists of a short N-terminal cytoplasmic domain, followed by a single transmembrane helix, and a large C-terminal extracellular domain that contains the active site (Fig. 2C) [18]. The variants of *MME* may cause the loss of *MME* expression. The *MME* mutations of c.1342 C>T (p.R448*) and c.1416+2T (p.V440_K472del) are located in the N-terminal peptidase M13 domain, and mutations of c.2122 A>T (p.K708*), c.2071_2072delinsTT (p.A691L) and c.2027 C>T (p.P676L) are located in the C-terminal peptidase M13 domain. Meanwhile, the variants p.R448* and p.V440_K472del are possibly associated with the loss of enzyme function of N-terminal and C-terminal peptidase of M13, and variant p.K708* may only have an impact on N-terminal or C-terminal peptidase of M13. The heterozygous *MME* variants, c.1342 C>T&c.2071_2072delinsTT and c.1416+2T>C&c.2027 C>T, and homozygous *MME* variant c.2122 A>T could cause both the loss of enzyme function of C-terminal peptidase of M13. Factually, previous studies had proved that loss of function might be the cause of *MME*-related dHMN or CMT2 [7, 9, 11].

MME encodes NEP, which exists in the central and peripheral nervous systems and can catalyze cleavage of bradykinin, substance P and neurotensin peptides [5, 19]. It may play an important role in neuronal function, for *MME* knockout mice showed enhanced aggressive behaviors, altered locomotor activities [20], and were more sensitive to heat and mechanical stimuli than wild-type mice [21]. It was hypothesized that the loss of function in *MME* mutations may cause demyelination and axonal degeneration by impairing Schwann cell–axonal interactions in late-onset CMT2 [7]. And in dHMN, the loss of *MME* expression might affect normal degradation of peptides and break the well-being of peripheral motor nerve. Furthermore, NEP is the major Aβ degrading enzyme in the nervous system and has been well-studied in Alzheimer's disease [6]. On the one hand, *MME* knockout or inhibition in animal models caused the accumulation of Aβ peptides in the brain [22], and NEP expression and activity were decreased in elderly AD patients according to a recent meta-analysis [23]. On the other hand, learning abilities were not reduced and Aβ deposits could not be detected in *MME* knockout mice [24]. Besides, there is no obvious evidence of early-onset Alzheimer's disease in *MME*-related CMT2 and dHMN patients [7]. Therefore, *MME* mutations or NEP deficiency alone, without the absence of other Aβ degrading enzymes, may be not sufficient to cause Aβ deposits and early-onset Alzheimer's disease [25].

Conclusion

Our study reported a "novel" nonsense mutation and a missense variant of autosomal recessive late-onset dHMN, which expanded the genetic spectrum of dHMN.

Acknowledgements

The authors thank the participating families for their assistance.

Author contributions

Bentuo Zhang wrote the main manuscript text. Qiang Gang, Kang Du and Lingchao Meng contributed the conception of the study. Baogang Huang, Haohao Wu and JunsuYang contributed to the acquisition of data. Zhenyu Li and Xujun Chu prepared Figs. 1 and 2. All authors reviewed and approved the final manuscript.

Funding

The study was supported by Yunnan Provincial Department of Education Science Research Funding.

Data availability

The details of the variant c.2122 A>T (p.K708*) analyzed in this study have been deposited into the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) with the accession number VCV003064216.1.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the Ethic Committee of Qujing First People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Written informed consents for publication of identifying images or other personal or clinical details were obtained from all participants and patients.

Received: 2 April 2024 / Accepted: 21 August 2024
Published online: 04 September 2024

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