Abstract. An aberrant elongated tract of glutamine residues (polyQ) in proteins induces multiple diseases treated in the clinic. In our previous study of progressive myoclonic epilepsy (PME), using whole-exome sequencing, a mutant Cav2.1 protein with an aberrant elongated polyQ tract was identified in PME patients. To investigate the molecular mechanism and cell biology of this aberrant elongated polyQ tract, wild-type Cav2.1 with 13 polyQ repeats (Cav2.1 wt-Q13) and mutant-type Cav2.1 with 26 polyQ repeats (Cav2.1 mt-Q26) were prepared and introduced into human SH-SY5Y neuroblastoma cells. Using a WST-1 assay, it was revealed that Cav2.1 mt-Q26 markedly suppressed the proliferation of the SH-SY5Y cells, a result not observed for the Cav2.1 wt-Q13-transfected cells. It was also revealed that Cav2.1 mt and its truncated molecules suppressed cell proliferation by inducing apoptosis rather than arresting the cell cycle. Further investigations indicated a nuclear translocation phenomenon associated with the Cav2.1 mt molecules. Mechanistically, it was revealed that the Cav2.1 mt molecules activated the Bcl-2/Bax, caspase-3 and poly ADP-ribose polymerase (PARP) apoptotic pathways. The present study may provide new insights for interpreting the pathogenesis of PME and the relationship among polyQ, CACNA1A gene mutations and PME.

Introduction

The CACNA1A gene, located on chromosome 19p13, encodes the pore-forming α1A subunit of human neuronal voltage-gated Cav2.1 (P/Q-type) calcium channels (1,2). Mutations in the CACNA1A gene cause several autosomal-dominant neurological disorders and clinical symptoms/phenotypes such as migraines, spinocerebellar ataxia, familial hemiplegic migraine (FHM), and episodic ataxia (EA) (3-6).

In our previous study, using whole-exome sequencing, a homozygous variant of Cav2.1 was identified in a three-generation consanguineous Chinese Han family with progressive myoclonic epilepsy (PME), which is a heterogeneous neurodegenerative disorder (7). The mutant Cav2.1 was revealed in two siblings but not in fifty normal donors, indicating the mutation gene spectrum of CACNA1A can be used as a valuable predictor for PME.

A repeated polymorphic cytosine-adenine-guanine (CAG) mutation was also identified in the PME patient samples that encodes an elongated tract of glutamine residues in the C-terminus of Cav2.1. It has been suggested that trinucleotide repeat expansion in the CAG in protein coding regions in the genome may cause polyglutamine (polyQ) disease, which induces a set of dominantly inherited neurodegenerative disorders including Huntington's disease (HD), spinocerebellar ataxia, type 1/2/3/6/7 (SCA-1/2/3/6/7), dentatorubropallidoluysian atrophy (DRPLA), Machado-Joseph disease (MJD) and spinobulbar muscularatrophy (8,9).

Although polyQ diseases are well known clinically, their molecular mechanism and cell biological behavior are still unclear, especially in the context of PME. To address this issue, in the present study, a wild-type (wt) human Cav2.1 (Cav2.1 wt) with 13 repeats of CAG as well as a mutant-type (mt) Cav2.1 (Cav2.1 mt) with 26 repeats of CAG in the C-terminus of this protein were constructed and delivered into cells of the SH-SY5Y neuroblastoma cell line, which is always used as the model cell line for investigations of human nervous system diseases (10-12). The results revealed that the forced expression of Cav2.1 mt significantly inhibited SH-SY5Y cell proliferation by inducing apoptosis. By constructing a series of truncated Cav2.1 mt molecules and a Cav2.1 mt-GFP fusion molecule, a marked nuclear translocation phenomenon was revealed. Furthermore, it was observed that Cav2.1 mt may activate apoptosis-relevant factors, namely, Bcl-2/Bax, caspase and PARP, and thus induces cell apoptosis. The present study clearly revealed the apoptosis-inducing effects of polyQ.
mutations in human nerve cells, a finding that may provide a new insight useful for interpreting the pathogenesis of and developing new therapeutic targets for PME.

Materials and methods

Cell culture. Human SH-SY5Y neuroblastoma cells were kindly provided by the Stem Cell Bank, Chinese Academy of Sciences (cat. no. SCSP-5014) and were authenticated by STR profiling provided by the Stem Cell Bank, Chinese Academy of Sciences. The cells were routinely maintained in Dulbecco’s modified Eagle’s medium (DMEM; GE Healthcare Life Sciences) supplemented with 10% fetal calf serum (FCS; GE Healthcare Life Sciences), 100 µg/ml streptomycin and 100 U/ml penicillin (HyClone; GE Healthcare Life Sciences). The cells were routinely serum-starved for 24 h before use. SH-SY5Y cells were cultured at 37°C in a humidified incubator containing 5% CO₂. The cell culture medium was changed every two days. Upon reaching 80% confluence, the cells were digested with 0.25% trypsin (GE Healthcare Life Sciences) for 1 min at 37°C.

Plasmid construction. The DNA molecules expressing Cav2.1 wt (Q13, 13 CAG repeats) and Cav2.1 mt (Q26, 26 CAG repeats) were synthesized by Sangon Biotech Co., Ltd. The first CAG starts from the 6955 site of CACNA1A cDNA (NM_023035.2). DNA molecules were cloned into pcDNA3.1.neo vector with BamHI and XbaI restriction enzyme sites. The DNA expressing a series of CACNA1A deletion mutants (Cav2.1dm) were synthesized by PCR and cloned into pcDNA3.1.neo vector with BamHI and XbaI. The primer sequences of the Cav2.1dm were as follows: Cav2.1N forward, 5’-CAA GGG ATC CGC CAC CAT GGC CCG GCC CCG TCTCGGAGAC-3’ and reverse, 5’-GCCCTCTTTAGTTAG GCCCTAGGATCACAGG-3’; Cav2.1dm1 forward, 5’-CAA GGGATCCGCCACATGCAGGTGTTTGGTTAACATTG-3’ and reverse, 5’-GCCCTCTTAGTTAGCAACAATATCG TCAC-3’; Cav2.1dm2 forward, 5’-CAAGGGATCCGCCAC CATGAAGCTTCAGCCCTCGG-3’ and reverse, 5’-GCC CCTCTTAGGATCACACATCTCAGTCAC-3’; Cav2.1C forward, 5’-CAAGGGATCCGCCACCATGCGGTGCG GCCAGCCGGGC-3’ and reverse, 5’-GCCCTCTTAGTTAG CACAACATGCTCAGTCAC-3’. The DNA sequences of Cav2.1dm3, Cav2.1dm4 and Cav2.1dm5 were synthesized by Sangon Biotech Co., Ltd. and cloned into the same vector via BamHI and XbaI restriction enzyme sites. To ascertain the cellular distribution of Cav2.1dm, GFP was fused to the C-terminus of Cav2.1N. The plasmid expressing Cav2.1dm3G fusion protein was constructed.

Polypeptide structure prediction. The 3D structure of polypeptide was built using an online tool (http: //zhanglab.ccb.med.umich.edu/).

Transfection. According to the manufacturer’s instructions, 2 µg of each of the aforementioned plasmids and the empty vector (EV, control) were transfected into SH-SY5Y cells using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). All the cellular experiments were carried out at or after 48 h post-transfection.

RNA extraction, reverse transcriptional reaction and real-time quantitative PCR (qPCR). Total RNA was isolated using EasyPureRNA Purification Kit (cat.no. ER701; BeijingTransGen Biotech Co., Ltd.). RNA (1,000 ng) was reverse-transcribed with TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (cat. no. AT341; Beijing TransGen Biotech Co., Ltd.). qPCR was conducted based on the instructions of RealStar Green Fast Mixture (GenStar). Primers for qPCR are listed as follows: Forward primer for Cav2.1 wt, Cav2.1 mt, Cav2.1N, and Cav2.1dm4 is 5’-AAGGATCCGAA CATTGACAG-3’ and reverse primer is 5’-GTTGCTTGTTACCA GATGTTGAG-3’; forward primer for Cav2.1dm1, Cav2.1dm2, Cav2.1dm3, Cav2.1C and Cav2.1dm5 is 5’-GACTCCCCCA ACGGCTACTAC-3’ and reverse primer is 5’-CCACAATATCA TCGTCACTCTCG-3’. qPCR was performed on ABI 7500 (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the PCR program consisted of 10 sec at 95°C, 40 cycles at 95°C for 5 sec and 60°C for 35 sec. The relative expression of the aforementioned target gene was calculated based on the 2⁻ΔΔCq method (13).

Cell growth assay. At 48 h post-transfection, SH-SY5Y cells were trypsinized and seeded into a 96-well plate at a density of 5×10³ cells/well and cultured for another 48 h. Cell proliferation was determined using WST-1 cell proliferation reagent (Roche Applied Science). According to the manufacturer’s instructions, 20 µl WST-1 was added to 200 µl cell culture medium and incubated at 37°C in the dark for 2.5 h. The absorbances at 450 and 630 nm were assessed by a microplate reader (BioTek Instruments). The final OD (optical density) was designated as OD450-OD630-OD_blank⁻.

Cell cycle and apoptosis assay. At 72 h post-transfection, at least 2×10⁵ cells were harvested and washed with phosphate-buffered saline (PBS) three times. For cell cycle analysis, cell pellets were resuspended in 200 µl PBS containing 10 µg/ml propidium iodide (PI; Sigma-Aldrich; Merck KGaA) and incubated for 30 min in the dark at room temperature. For cell apoptosis, the transfected cells were stained with an Annexin V-FITC/PI Apoptosis Detection kit (BD Biosciences) for 15 min in the dark and analyzed using a flow cytometer (FACScan; BD Biosciences) with FlowJo analysis software (version 10.0; FlowJo LLC). The cells in the quadrants represented the different cell states as follows: Late-apoptotic cells were presented in the upper left quadrant, viable cells were presented in the lower left quadrant, and early apoptotic cells were presented in the lower right quadrant.

Protein extraction. Protein was extracted from the whole cell, cell membrane, or cell nucleus of the SH-SY5Y cells, respectively. The whole cell protein was extracted with a Protein Extraction kit (cat. no. KGP2100; Nanjing KeyGen Biotech. Co., Ltd.). Cell membrane protein and nucleoprotein were extracted with a Cell Membrane Protein/Cytoplasmic Protein/Nucleoprotein Extraction kit (cat. no. KGBSP002; Nanjing KeyGen Biotech. Co., Ltd.).

Western blotting. Equal amounts of 25 µg protein from each sample were loaded to 10% SDS polyacrylamide gel and separated by electrophoresis. Then, proteins were transferred to PVDF membranes (EMD Millipore). The membranes were incubated in 5% BSA (cat. no. E661003; Sangon
Biotech Co., Ltd.)/TBST solution at 37°C for 1 h and then washed in TBST 3 times for 5 min. The membranes were then incubated with primary antibodies at 4°C overnight. The primary antibodies were: Anti-Cav2.1 N-terminus (product code ab191140; recognizes Human CACNA1A aa 430-447; 1:1,500); anti-Cav2.1 C-terminus (product code ab181371; recognizes Human CACNA1A aa 2050-2150; 1:15,000); anti-Bel-2 rabbit polyclonal antibody (product code ab59348; 1:15,000); and anti-Bax (product code ab32503; 1:1,500; all from Abcam); anti-cleaved caspase-3 (product no. 9664; 1:1,000; Cell Signaling Technology, Inc.); anti-cleaved poly ADP-ribose polymerase (PARP) (product code ab32064; 1:1,500); anti-GFP (product code ab6556, 1:3,000); and anti-β-actin (product code ab8226, 1:3,000; all from Abcam). After washing in TBST 3 times for 5 min, the membranes were blocked with 5% powdered milk in TBST buffer (Sangon Biotech Co., Ltd.) plus 0.02% sodium azide (Sigma-Aldrich; Merck KGaA) for 1 h at RT. After washing 3 times for 5 min, the cells were incubated with TBST/0.5% BSA diluted Alexa Fluor 555 conjugate goat anti-rabbit IgG secondary antibody (cat. no. A-21428; Thermo Fisher Scientific, Inc.) at a concentration of 4 µg/ml (according to the manufacturer's instructions) for 45 min at RT.

To visualize the intracellular location of Cav2.1dm3G, cell nuclei were stained with DAPI. According to the manufacturer's instructions, SH-SY5Y cells expressing Cav2.1dm3G were washed with PBS 2 times for 2 min and 1.5 ml DAPI (Beyotime Institute of Biotechnology) was added to the wells. Then the cells were maintained at room temperature for 5 min.

An Olympus IX83 fluorescence microscope (Olympus Corporation) was used to observe the cellular morphology and fluorescence.

Statistical analysis. Statistical analysis was conducted with SPSS 17.0 software (SPSS, Inc.). All data were expressed as the mean ± standard deviation. All of the experiments were performed in triplicate and repeated at least three times. Significance was determined using Student's t-test or one-way analysis of variance (ANOVA) followed by LSD or Tukey's post hoc test. P<0.05 and P<0.01 were considered to indicate statistically significant differences (*P<0.05 and **P<0.01 as indicated in the images and figure legends).

Results

Cav2.1 mt with 26 polyQ repeats suppresses the proliferation of the SH-SY5Y cells. The DNA molecules expressing Cav2.1 wt and Cav2.1 mt were synthesized and confirmed by Sanger sequencing (Fig. 1A and B). After the two expressing plasmids, pcDNA3.1-Cav2.1 wt and pcDNA3.1-Cav2.1 mt, were delivered into SH-SY5Y cells and confirmed by qPCR (Fig. 1C) cell proliferation was analyzed. As revealed in Fig. 1D, compared with the Cav2.1 wt-Q13 and the EV transfectants, the forced expression of Cav2.1 mt-Q26 significantly decreased the cell proliferation of the SH-SY5Y cells (P<0.01).

Biological function of the truncated Cav2.1 mt molecules. It has been reported that Cav2.1 truncating mutation is associated with neurological disorders (14). Herein, to address whether the truncated Cav2.1 mt molecules affect the biological functions...
of the SH-SY5Y cells, a series of CACNA1A gene deletion mutations were prepared and the corresponding expressing plasmids that are schematically represented in Fig. 2A, were constructed. Cav2.1N is the a1 to aa2312 on the N-terminus of Cav2.1 containing no polyQ; Cav2.1dm1 is the aa1716 to aa2525 of Cav2.1 mt containing 26 polyQ repeats; Cav2.1dm2 is the aa2127 to aa2525 of Cav2.1 mt containing 26 polyQ repeats; Cav2.1dm3 is the aa2319 to aa2525 of Cav2.1 mt, starting from the first Q and containing 26 polyQ repeats; Cav2.1C is the aa2345 to aa2525 on the C-terminus of Cav2.1 containing no polyQ. The approximate molecular weight (kDa) of each deletion mutation was calculated by an online tool (http://www.detaibio.com/sms2/protein_mw.html). SH-SY5Y cells were then transfected with all the aforementioned plasmids and the transfection efficacy was confirmed by qPCR (Fig. 2B). The cell proliferation of all the transfected cells were detected by WST-1 and the result indicated that Cav2.1N and Cav2.1C, containing no polyQ repeat did not affect the cell proliferation of the SH-SY5Y cells. Conversely, the forced expression of Cav2.1dm1, Cav2.1dm2 and Cav2.1dm3, containing 26 polyQ repeats significantly impaired cell proliferation. \( P<0.01 \), a significant difference in cell proliferation was observed between EV/Cav2.1N/Cav2.1C and Cav2.1dm1/Cav2.1dm2/Cav2.1dm3, and Cav2.1dm1/Cav2.1dm2 and Cav2.1dm3. NS, no difference of cell proliferation was observed among EV, Cav2.1N and Cav2.1C, and Cav.1dm1 and Cav2.1dm2 (Tukey’s test). (D) Comparison of the proliferation of the SH-SY5Y cells transfected with Cav2.1dm3 and its two variations. The result indicated that only those molecules composed with integrity Q26 and C-terminus of Cav2.1 harbored cytotoxicity (Tukey’s test). (E) Partial amino acid sequences of Cav2.1 wt-Q13 and Cav2.1dm3. (F) Comparison of the 3D structure of Cav2.1 wt and Cav2.1dm3. White arrows indicate the differences on the secondary structure between Q13 and Q26. \( P<0.01 \). Cav2.1mt, mutant-type Cav2.1; Cav2.1 wt, wild-type Cav2.1; EV, empty vector; NS, no significance.
the cell proliferation of the SH-SY5Y cells. Conversely, the forced expression of Cav2.1 dm1, Cav2.1 dm2 and Cav2.1 dm3, containing 26 polyQ repeats, significantly impaired cell proliferation (Fig. 2C, P<0.01). Notably, the Cav2.1 dm3 transfectants had the lowest proliferation (Fig. 2C).

Since the Cav2.1 dm3 molecule is composed by Q26 and Cav2.1C, and Cav2.1C itself does not affect cell proliferation, therefore it was speculated that Q26 plays a critical role in the Cav2.1-induced cell growth inhibition. To ascertain our hypothesis, another two plasmids were further constructed. Cav2.1 dm4, aa2127 to aa2344 of Cav2.1 mt, contains 26 polyQ repeats and ends at the last Q, with no Cav2.1 C-terminus. Cav2.1 dm5 is the reversed arrangement of Q26 (aa2319-2344) and Cav2.1C (aa2345-2525). After transfection, it was revealed that neither Cav2.1 dm4 nor Cav2.1 dm5 affected the cell growth of the SH-SY5Y cells (Fig. 2D, P>0.05). These data indicated that: i) The expanded mutation of the polymorphic Q repeat tract, e.g., from 13 repeats to 26 repeats, has cytoxicity and impairs the cell growth of the SH-SY5Y cells; and ii) the Q26 tract must be linked with the Cav2.1 C-terminus in a cis-arrangement to exert its biological function.

It was surmised that the structure of the glutamine-expanded Cav2.1 is unstable, and the mutant protein cleaves and generates truncated molecules. Therefore the 3D structure of the truncated Cav2.1 beginning with Q13 and Q26 (Fig. 2E) was compared using an online tool (http://zhanglab.ccmb.med.umich.edu/). Because the website has set a limit on the amino acid number, the structure of the truncated Cav2.1 beginning with Q26 and Q50 was also constructed and compared (Fig. 2E).

Figure 3. The forced expression of Cav2.1 mt molecules do not affect the cell cycle distribution of the SH-SY5Y cells. (A) The cell cycle was analyzed by flow cytometry and the results indicated that compared with the EV and Cav2.1 wt groups, Cav2.1 mt and Cav2.1 dm3 transfectants exhibited no difference in cell cycle distribution. (B) Statistical result of the cell cycle distribution. No difference in cell cycle distribution was observed among EV, Cav2.1 wt, Cav2.1 mt and Cav2.1 dm3 (Tukey’s test). Cav2.1 mt, mutant-type Cav2.1; Cav.1 wt, wild-type Cav2.1; EV, empty vector; NS, no significance.

Figure 4. The forced expression of Cav2.1 mt and Cav2.1 dm3 induces cell apoptosis in SH-SY5Y cells. (A) The flow cytometric result indicated that compared with the Cav2.1 wt and EV groups, the forced expression of Cav2.1 mt and Cav2.1 dm3 significantly induced cell apoptosis in SH-SY5Y cells. **P<0.01, a significant difference in cell apoptosis was observed between EV/Cav2.1 wt and Cav2.1 mt/Cav2.1 dm3, and Cav2.1 mt and Cav2.1 dm3. NS, no difference in cell apoptosis was observed between EV and Cav2.1 wt (Tukey’s test). (B) Cell morphology also revealed that Cav2.1 mt and Cav2.1 dm3 markedly induced cell apoptosis. Cav2.1 mt, mutant-type Cav2.1; Cav.1 wt, wild-type Cav2.1; EV, empty vector; NS, no significance.
acid quantity, the 3D structures of the two molecules with 195aa and 208aa are only presented. As revealed in Fig. 2F, the structures of the molecules with Q13 and Q26 at the N-terminus of the truncated Cav2.1 were quite different.

Truncated Cav2.1 does not affect the cell cycle distribution of the SH-SY5Y cells. To elucidate the anti-proliferation mechanisms of the truncated Cav2.1, a cell cycle assay was performed using flow cytometry. However, no variation of cell cycle distribution was observed among the EV, Cav2.1 wt, Cav2.1 mt and Cav2.1dm3 transfectants (Fig. 3, P>0.05).

Truncated Cav2.1 promotes apoptosis of the SH-SY5Y cells. Subsequently, a cell apoptosis assay was performed using flow cytometry. As revealed in Fig. 4, compared to the Cav2.1 wt and EV transfection groups, the forced expression of Cav2.1 mt and Cav2.1dm3 in SH-SY5Y cells significantly induced apoptosis. The apoptotic rate of the Cav2.1dm3 group was 30.5%, significantly higher than that of 14.53% in the Cav2.1 mt group (Fig. 4A, P<0.01). The cell morphology also indicated the increased apoptosis in the Cav2.1dm3 transfectants (Fig. 4B).

Truncated Cav2.1 molecules are translocated to the nucleus of the SH-SY5Y cells. To gain deeper insight into the role of the truncated Cav2.1 mt molecules in cell apoptosis, a Cav2.1dm3 and GFP fusion molecule was constructed. To avoid impairing the Q26 bioactivity, GFP was fused to the C-terminus of Cav2.1dm3, and named Cav2.1dm3G (Fig. 5A). After the plasmid was delivered into SH-SY5Y cells, the expression of Cav2.1dm3G was detected by western blotting.
As controls, the expression of Cav2.1 wt and Cav2.1 mt in the transfectants were also detected by western blotting, concurrent. As revealed in Fig. 5B, the western blot results revealed that the Cav2.1 wt molecules were mainly located on the cell membrane (87.0%, the upper right panel, P<0.01), Cav2.1 mt molecules were located both on the cell membrane and in the cell nucleus (46.7% vs. 53.3%, the left lower panel, P<0.05), whereas most Cav2.1dm3G molecules were located in the cell nucleus (85.9%, the right lower panel, P<0.01).

A cell immunofluorescence staining experiment was also performed and it was observed that the Cav2.1 wt molecules were distributed on the cell membrane, whereas the GFP fluorescence of Cav2.1dm3G molecules was mainly distributed inside the cell nucleus (Fig. 5C). These data indicated that the truncated Cav2.1 mt molecules were translocated to nucleus, increasing apoptosis of the SH-SY5Y cells.

Truncated Cav2.1 mt induces cell apoptosis via the Bcl-2/Bax, caspase-3 and PARP pathway. It has been reported that polyQ interacts with the mitochondrial apoptotic pathway and caspase apoptotic proteins to exert cytotoxicity (15). Therefore, the expression of Bcl-2, Bax, cleaved caspase-3 and cleaved PARP were detected. Western blot results revealed that the Bcl-2/Bax ratio was markedly decreased in Cav2.1 mt.
and Cav2.1dm3 transfectants (Fig. 6, upper left and upper right panel, P<0.01). The caspase-3 and PARP cell apoptotic pathways were also activated (Fig. 6 upper left panel, lower left/right panel, P<0.01).

Discussion

PME comprises a group of rare neurodegenerative disorders characterized by ataxia, myoclonus, seizures, and progressive cognitive impairment (16). The occurrence and development of PME is always accompanied by certain types of gene mutations. Tonin et al identified a newly discovered synonymous c.363A>G (Gly82Gly) mutation in the β-glucosidase (GBA) gene in a type 3 Gaucher disease patient that causes the loss of an exon splicing enhancer (17). He et al revealed a novel c.995-1G>A homozygous splicing mutation in the SCARB2 gene in two PME patients that leads to loss of function of the SCARB2 protein (18).

In recent years, an increasing number of mutations in the CACNA1A gene have been reported as causative factors for several types of neurological disorders. Using next-generation sequencing, Grieco et al identified an R2157G missense variant in a Moroccan hemiplegic migraine patient and an I1512T missense variation in patients with FHM (6). Using whole-exome sequencing, Khaiboullina et al identified an R583Q missense mutation in patients with FHM (5). Algahtani et al summarized that there are more than 170 disease-causing variants of CACNA1A, including mutations with small deletions, gross deletions, missense variants, nonsense variants, splice variants, small insertions, complex rearrangements and repeat variations (19).

In a previous study, using whole-exome sequencing, we identified a homozygous variant in PME patients: a repeated CAG mutation of Cav2.1 inducing cell apoptosis. Sanchez et al reported that the polyglutamine repeat (Q79)/caspase 8 axis-induced cell death was critical in Huntington's disease (25). Tien et al demonstrated that the polyglutamine repeat (Q26 and Q78) may decrease the mRNA level of Bcl-2 in Machado-Joseph disease cells (26). In the present study, it was revealed that the truncated Cav2.1 mutant molecule induced cell apoptosis through the activation of the Bcl-2/Bax, caspase-3 and PARP pathways. The production of PAR chains by PARPs is one of the first steps in the process of DNA damage repair (27). In addition, the increased level of cleaved caspase-3 can lead to cleaved PARP, which is an inactive form, thereby disrupting DNA repair and resulting in apoptosis (28). Considering the relationship among Bcl-2/Bax, caspase-3 and PARP, the present results are reasonable. However, since few investigations have focused on the correlation between polyQ and PARP, to support our conclusion, further experiments are required.

In summary, in the present study, it was demonstrated that an aberrant elongated tract of polyQ in the Cav2.1 protein may proteolytically generate a short fragment that is translocated to the nucleus, inducing cell apoptosis in human SH-SY5Y cells (Fig. 7). The present study may provide new insights for the interpretation of the pathogenesis of PME and for the relationship among polyQ, CACNA1A gene mutation and PME.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

DM and YL contributed to the conception and design of the study. JS and XS contributed to the acquisition, analysis and interpretation of data. JS drafted the manuscript. XS and ZL analyzed the data and was involved in performing the experiments. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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