Mutant Huntingtin Causes a Selective Decrease in the Expression of Synaptic Vesicle Protein 2C

Chaohua Peng¹ · Gaochun Zhu¹ · Xiangqian Liu¹ · He Li^{1,2}

Received: 25 January 2018 / Accepted: 24 March 2018 / Published online: 30 April 2018
© Shanghai Institutes for Biological Sciences, CAS and Springer Nature Singapore Pte Ltd. 2018

Abstract Huntington's disease (HD) is a neurodegenerative disease caused by a polyglutamine expansion in the huntingtin (Htt) protein. Mutant Htt causes synaptic transmission dysfunctions by interfering in the expression of synaptic proteins, leading to early HD symptoms. Synaptic vesicle proteins 2 (SV2s), a family of synaptic vesicle proteins including 3 members, SV2A, SV2B, and SV2C, plays important roles in synaptic physiology. Here, we investigated whether the expression of SV2s is affected by mutant Htt in the brains of HD transgenic (TG) mice and Neuro2a mouse neuroblastoma cells (N2a cells) expressing mutant Htt. Western blot analysis showed that the protein levels of SV2A and SV2B were not significantly changed in the brains of HD TG mice expressing mutant Htt with 82 glutamine repeats. However, in the TG mouse brain there was a dramatic decrease in the protein level of SV2C, which has a restricted distribution pattern in regions particularly vulnerable in HD. Immunostaining

revealed that the immunoreactivity of SV2C was progressively weakened in the basal ganglia and hippocampus of TG mice. RT-PCR demonstrated that the mRNA level of SV2C progressively declined in the TG mouse brain without detectable changes in the mRNA levels of SV2A and SV2B, indicating that mutant Htt selectively inhibits the transcriptional expression of SV2C. Furthermore, we found that only SV2C expression was progressively inhibited in N2a cells expressing a mutant Htt containing 120 glutamine repeats. These findings suggest that the synaptic dysfunction in HD results from the mutant Htt-mediated inhibition of SV2C transcriptional expression. These data also imply that the restricted distribution and decreased expression of SV2C contribute to the brain region-selective pathology of HD.

Keywords Synaptic vesicle protein 2C · Huntington's disease · Basal ganglia · Hippocampus

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12264-018-0230-x>) contains supplementary material, which is available to authorized users.

✉ Xiangqian Liu
liu_xiangqian@hotmail.com

✉ He Li
heli@mails.tjmu.edu.cn

¹ Department of Histology and Embryology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

² Key Laboratory of Ministry of Education of China for Neurological Disorders, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Introduction

Huntington's disease (HD) is an autosomal dominant inherited neurological disorder characterized by progressive motor deficits, cognitive decline, and psychiatric symptoms. It is caused by a pathological expansion of CAG trinucleotide repeats in exon 1 of the HD gene, resulting in the translation of a mutant form of huntingtin protein (mutant Htt) with an expanded polyglutamine domain in the N-terminal region [1]. A normal *Htt* gene has 6–35 CAG repeats, and a mutant *Htt* with 40 or more repeats always causes HD. At the population level, there is an inverse correlation between CAG repeat length and the age of onset of HD [2, 3].

HD is regarded as a disorder that primarily affects the basal ganglia [4, 5]. The basal ganglia are a set of subcortical nuclei critical for motor, cognitive, and behavioral functions. The striatum (putamen and caudate nucleus), globus pallidus [internal segment (GPi) and external segment (GPe)], and substantia nigra [pars compacta (SNc) and pars reticulata (SNr)] are components of the basal ganglia. The striatum is the main input structure; it receives excitatory glutamatergic inputs from multiple regions and projects information to other basal ganglia nuclei [6]. The GPe is a major hub that plays an important role in integrating and conveying information in the basal ganglia macrocircuit [7–10]. The GPi and SNr are the output nuclei [11, 12]. The SNc is composed of closely-packed dopaminergic neurons that control movement through their projections to the striatum [13].

It was generally believed that the neurological symptoms of HD are mainly caused by neurodegeneration and neuronal loss in the basal ganglia, especially the striatum [14]. However, accumulating evidence has shown that subtle alterations in the synaptic function of the basal ganglia circuitry underlie the early symptoms of HD, and pharmacological interventions that target early synaptic disturbances have been reported to reverse neuronal dysfunction and delay progression to neurodegeneration [15–17].

Cognitive deficits are an early clinical manifestation of HD and can occur 10 years prior to the onset of motor symptoms [18, 19]. Annual cognitive decline has been shown to be a robust marker of HD progression [20]. Studies in patients and animal models have demonstrated that the early cognitive deficits in HD are associated with not only dysfunction of the basal ganglia circuitry [21], but also hippocampal deficits. The hippocampus is a key region for cognition and has been reported to show some of the earliest signs of neuropathology and synaptic impairments in HD [22–27].

Growing evidence indicates that mutant Htt causes synaptic transmission dysfunctions that lead to early HD symptoms by disrupting the normal expression of synaptic proteins such as synaptophysin, SNAP-25, and Rab3A [28–30]. Synaptic vesicle proteins 2 (SV2s), a family of synaptic vesicle proteins including 3 members, SV2A, SV2B, and SV2C, plays important regulatory roles at synapses, and is essential for normal neurotransmission [31, 32]. However, the role of SV2s in HD neuropathology is not clear, and the effect of Htt mutations on the expression of SV2s has not yet been reported.

Here, we investigated whether the expression levels and pattern of SV2s are affected by mutant Htt in the brains of N171-82Q HD transgenic (TG) mice and Neuro2a mouse neuroblastoma cells (N2a cells) expressing mutant Htt.

Materials and Methods

Animals

The N171-82Q HD TG mice (expressing the N-terminal 171 amino-acid fragment of human huntingtin with 82 polyglutamines; TG mice) [33] and the control mice (wild-type littermates; WT mice) were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were housed in a temperature- and humidity-controlled, specific pathogen-free environment with a 12 h light/dark cycle and free access to food and water. All animal experiments were conducted in accordance with the guidelines for animal research and approved by the Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, China.

Tissue Preparation

After deep anesthesia by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight), the mice were perfused *via* the ascending aorta with 50 mL of 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4), followed by 100 mL of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH 7.4). Then the brain was removed, post-fixed in the same fixative for 6 h at 4°C, cryoprotected with 30% sucrose in 0.1 mol/L PB at 4°C until it sank, quickly frozen, and cut into 25 μ m-thick sections on a cryostat (Leica, Nussloch, Germany). All sections were collected in ice-cold 0.01 mol/L PBS and kept at 4°C until use.

Immunohistochemical Staining and Immunostaining Density Analysis

Coronal sections were immunostained for SV2C isoform using the avidin-biotin-peroxidase complex method as previously described [34, 35]. Briefly, the sections were immersed in 3% hydrogen peroxide to quench endogenous peroxidase activity, then in blocking solution to prevent non-specific binding of the antibody to the tissues. Thereafter, the sections were incubated overnight at 4°C with rabbit anti-SV2C polyclonal antibody (1:500 dilution, ref. ab33892, Abcam, Cambridge, UK). They were then incubated with biotinylated goat anti-rabbit IgG (1:200 dilution, Vector Labs, Burlingame, CA) and avidin-biotin-peroxidase complex (1:100 dilution, Vector Labs) at room temperature for 2 h. Between incubation periods, the tissues were rinsed in 0.01 mol/L PBS. Finally, the immunoreactive products were visualized by incubating the sections with 0.02% DAB (Sigma-Aldrich, St. Louis, MO) and 0.005% hydrogen peroxide in 0.05 mol/L Tris-

HCl buffer. The sections were dehydrated, coverslipped, and then observed and photographed under a light microscope (Nikon BX-51, Tokyo, Japan). In negative controls, the primary antibody was omitted. The mean optical density (integrated optical density (IOD)/area) was measured to assess the level of SV2C expression in immunostaining-positive areas using Image-Pro Plus 6.0 software (Media Cybernetics Inc., Rockville, MD) [36, 37].

Cell Culture and Transfection

N2a cells (CCL-131, American Type Culture Collection, Manassas, VA) were maintained as monolayer cultures in 90% Dulbecco's modified Eagle's medium (Gibco-Invitrogen Co., Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (Gibco-Invitrogen Co.), 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C in a 5% CO₂ atmosphere in a humidified incubator.

The cells were transfected with pEGFP-exon1 Htt-20Q (Htt-20Q) and pEGFP-exon1 Htt-120Q (Htt-120Q) plasmids (kindly provided by Dr. Xiao-Jiang Li, Department of Human Genetics, Emory University School of Medicine, Atlanta, GA), which encode an amino-terminal fragment of Htt containing 20 (20Q) or 120 (120Q) glutamine repeats, respectively, with Lipofectamine 2000TM (Invitrogen) according to the manufacturer's protocol.

Western Blotting

The whole brain, striatum, or transfected N2a cells were lysed in ice-cold lysis buffer [50 mmol/L Tris (pH 7.4), 50 mmol/L NaCl, 1% Triton X-100, 1 mmol/L EDTA (pH 8.0)] with a protease inhibitor cocktail (1:1000, Sigma-Aldrich) and phenylmethylsulfonyl fluoride (100 µg/mL, Sigma-Aldrich), and then centrifuged at 12,000×g for 15 min at 4°C. The supernatant was collected, and the protein concentration was measured by BCA protein assay (Pierce, Rockford, IL). The extracted proteins were then separated on 10% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked in 5% skim milk in PBS for 1 h at room temperature and then incubated with the following antibodies overnight at 4°C: rabbit anti-SV2A (1:1000, ref ab32942, Abcam), rabbit anti-SV2B (1:1000, ref 14624-1-AP, Proteintech, Wuhan, China), rabbit anti-SV2C (1:1000, ref ab33892, Abcam), and mouse anti-γ-tubulin (1:5000, Sigma-Aldrich). The membranes were washed and then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. The labelled protein bands were detected using an enhanced chemiluminescence kit (Pierce). Images were captured using the ChemiDoc XRS+ system (Bio-Rad, Hercules, CA) and analyzed using Image-Pro Plus 6.0 software (Media Cybernetics Inc.).

RNA Extraction and Reverse Transcription-PCR (RT-PCR)

Total RNA was isolated from brain structures including the striatum and hippocampus and from transfected N2a cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The RNA obtained was then converted to cDNA with a Superscript first-strand synthesis kit (Takara, Tokyo, Japan). PCR reactions were performed with 50 µL PCR mix containing 25 µL of 2× master mix (Qiagen, Hilden, Germany), 2 µL of primer mix, 1 µL of cDNA, 1 µL of β-actin internal primer mix and 21 µL of deionized water under the following reaction conditions: one cycle of initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s and a single final extension step at 72°C for 3 min. The following primer sequences were used: mouse SV2A forward, 5'-TCGTCCTTCGTCCAGGGTAA-3', and reverse, 5'-ATGAA TCGTTTAAATGTGGGTAC-3' (amplified product length, 459 bp); mouse SV2B forward, 5'-CACTGTCTA CAGGATACCGC-3', and reverse, 5'-CCCACTATGACA ATCTGCTAG-3' (product length, 347 bp); mouse SV2C forward, 5'-TGAGATGCTTCAACTACCCAGTCAGG-3', and reverse, 5'-CCTTTTGCACATTCTAGTTAGCAG-3' (product length, 162 bp); mouse β-actin 1 forward, 5'-GTCGTACCACAGGCATTTGTGATGG-3', and reverse, 5'-GCAATGCCTGGGTACATGGTGG-3' (product length, 493 bp); mouse β-actin 2 forward, 5'-CGTTGACATCCGT AAAGACC-3', and reverse, 5'-ACAGTCCGCCTAGAAG CAC-3' (product length, 280 bp). The amplified PCR products were electrophoresed on 1% agarose gels containing 0.03% ethidium bromide. The results were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics Inc.).

Statistical Analysis

The results are presented as mean ± SD from at least three independent experiments. Statistical analyses were performed using Student's *t*-test or one-way ANOVA. Differences were considered significant at *P* < 0.05.

Results

The Protein Expression of SV2C, but not of SV2A and SV2B, Was Decreased in the Brains of HD TG Mice

Transcriptional dysregulation is an early and progressive event that has been hypothesized to be an important pathogenic mechanism in HD [38–45]. To explore whether

SV2 is involved in the early pathology of HD, we first compared the protein expression levels of three SV2 isoforms in the whole brain of HD TG and WT mice. The N171-82Q TG mouse expresses a human N-terminal-truncated Htt (the first 171 amino-acids) that contains 82 glutamines in neurons throughout the brain and is therefore a useful model for both presymptomatic and symptomatic preclinical trials [33, 46, 47]. Motor deficits in these mice begin at ~12 weeks and cognitive deficits at ~14 weeks [46, 48–50]. Thus, at 14 weeks, the mice exhibit the early stages of disease progression, and this age was therefore chosen for further experiments.

The results of western blot analysis showed that, while there were no significant changes in the protein levels of SV2A and SV2B, SV2C was expressed at markedly lower levels in the TG mice than in the WT controls (Fig. 1).

SV2C-Immunoreactivity Progressively Decreased in the Basal Ganglia and Hippocampus in HD TG Mice

To further clarify which brain areas exhibit a decrease in the expression of the SV2C protein, we performed immunohistochemical staining and immunostaining density analysis.

Consistent with previous reports [51–53], immunohistochemical staining of coronal mouse brain sections revealed a high intensity of SV2C immunoreactivity in restricted regions. The most prominent staining was in the basal ganglia, including the striatum and its projection areas, such as the globus pallidus and substantia nigra, as well as the hippocampus, including the stratum lucidum of CA3 and the polymorphic layer of the dentate gyrus (Fig. 2). Intriguingly, early and progressive atrophy has been detected using structural MRI scans in the striatum and hippocampus in N171-82Q mice [47]. The motor and cognitive deficits in HD have been associated with pathological changes in the frontostriatal circuitry and the hippocampus [47, 54, 55]. These findings reveal that the

localization of the SV2C protein in the brain extensively overlaps with regions known to be vulnerable in HD.

Next, SV2C-immunoreactivity in the basal ganglia and the hippocampus was compared between TG mice and their WT littermates. In the basal ganglia, the striatum is composed mostly of medium spiny neurons, which project to the globus pallidus and the substantia nigra. The mRNA of SV2C is expressed in the striatum, and the SV2C protein is mainly distributed in the globus pallidus and the substantia nigra [52]. Here, we measured the levels of SV2C protein in the globus pallidus and substantia nigra as well as the hippocampus. In N171-82Q mice, neuronal loss and brain atrophy in addition to mutant Htt-positive inclusions have been observed in the striatum and hippocampus by the time the mice are 16 weeks old [46, 54, 56, 57], and the life expectancy of these mice is 20–24 weeks [46, 58]. To determine the association between changes in SV2C protein expression and HD progression, we used 16- and 20-week-old in addition to 14-week-old mice.

Using immunohistochemical staining density analysis, we found that SV2C-immunoreactivity became progressively lower with age in the basal ganglia and hippocampus of TG mice than in the WT controls (Fig. 3), suggesting that mutations in the *Htt* gene decrease the SV2C protein levels in specific mouse brain regions during the early stage of HD and that this reduction becomes more profound as the illness progresses.

The mRNA Level of SV2C, but not of SV2A and SV2B, Progressively Declined in the Brains of HD TG Mice

Given that mRNA and protein expression levels need not necessarily share a linear and simple relationship [59, 60], we performed parallel experiments to assess the mRNA levels of SV2 genes and further validate their roles in transcriptional dysregulation mechanisms in HD.

Fig. 1 Protein expression levels of SV2A, SV2B, and SV2C in whole brains from 14-week-old TG and WT mice. **A** Western blots. **B** Statistical analysis of relative protein expression levels (* $P < 0.05$; $n = 3$ pairs of age-matched TG and WT mice).

Fig. 2 Localization of SV2C in mouse brain. Coronal sections were stained with an antibody specific for SV2C. The most prominent staining for SV2C was in the striatum, globus pallidus, substantia nigra, and hippocampus, including the stratum lucidum of CA3 and the polymorphic layer of the dentate gyrus. STR, striatum; GP, globus pallidus; SN, substantia nigra; Hipp, hippocampus; RSG, retrosplenial granular cortex; DpMe, deep mesencephalic nucleus; RN, red nucleus; SC, superior colliculus; AMY, amygdala; Hypo, hypothalamus; VTA, ventral tegmental area; scale bar, 1 mm.

RT-PCR analysis showed that, consistent with the results of western blot analysis, there were no significant changes in the mRNA levels of SV2A and SV2B, but the mRNA expression level of SV2C in the whole brain was markedly lower at 14 weeks of age in TG mice than in WT controls (Fig. S1). Furthermore, a progressive decrease in the SV2C mRNA level was also found in the striatum and hippocampus in the TG mice (Fig. 4). These results suggest that during the early stages and also during the progression of the disease, mutations in the *Htt* gene decrease the SV2C protein level by inhibiting its transcription.

The Expression Level of SV2C, but not of SV2A and SV2B, Was Progressively Inhibited in N2a Cells Expressing Mutant Htt

In vitro models are invaluable for integrating data collected *in vivo* [61, 62]. To further clarify the relationship between mutant Htt and SV2C expression, we transfected N2a cells with the pEGFP-exon1 Htt-120Q (Htt-120Q) plasmid to establish an HD cell model and with the pEGFP-exon1 Htt-20Q (Htt-20Q) plasmid to establish a control line of cells. At 24 h, 48 h, and 72 h after transfection, the cells were collected to assess SV2C expression.

As shown in Fig. 5A, the N2a cells stably expressed the EGFP-Htt-120Q and EGFP-Htt-20Q fusion proteins, and the former cells formed aggregates. Furthermore, using western blot and RT-PCR assays, we found that there were no significant changes in SV2A and SV2B expression (Fig. S2), but the expression of SV2C protein and mRNA became progressively lower after 48 h and 72 h in the N2a cells transfected with pEGFP-Htt-120Q than in the control

cells (Fig. 5B, C). These results support our *in vivo* findings in HD mice, further indicating that mutant Htt suppresses the protein expression of SV2C by inhibiting its transcription.

Discussion

HD is a neurological disorder caused by the abnormal expansion of a CAG repeat coding for polyglutamine in the Huntingtin protein. Longer CAG expansion is associated with an earlier onset of symptoms and faster disease progress [63]. Cognitive decline is the prevalent clinical manifestation, even in the prodromal phase, and subclinical changes in cognition can occur up to 15 years prior to motor symptoms [19]. The cognitive defects and disease progression are typically associated with synaptic dysfunction [21, 64]. Although most of the symptoms have classically been thought to be caused by the neurodegeneration and progressive neuronal loss, increasing evidence indicates that synaptic dysfunction underlies the early symptoms and pathogenesis of HD [17, 64–73]. While most previous studies have concentrated on basal ganglia circuits, in which correlations between basal ganglia synaptic deficits and cognitive impairments have been demonstrated [74–78], some studies have also investigated the role of the hippocampus in HD. The hippocampus consists of the cornu ammonis (or hippocampus proper) and the dentate gyrus, and plays important roles in many cognitive processes, such as learning, memory, and decision-making [79]. Results from rodent models and patient studies have shown that hippocampal dysfunctions are an

A key factor affecting synaptic function is the presynaptic release of neurotransmitters by Ca^{2+} -triggered

Fig. 4 RT-PCR analysis of SV2C mRNA levels in the basal ganglia and hippocampus of TG and WT mice. The expression levels of SV2C mRNA in the striatum (A) and hippocampus (B) became significantly and progressively lower in TG mice than in WT control mice (* $P < 0.05$, ** $P < 0.01$, $n = 5$ /group).

synaptic vesicle exocytosis. At least five different types of synaptic vesicle proteins are involved in regulating the exocytotic process, one of which is SV2 [31, 32]. SV2 is a component of all vertebrate synaptic vesicles and plays a critical role in the proper functions of the nervous system by regulating synaptic vesicle trafficking and the Ca^{2+} concentration-dependent release of neurotransmitters [31, 84, 85]. Thus, we hypothesized that SV2 plays a role in HD-associated synaptic and cognitive dysfunction.

N171-82Q TG mice have been extensively studied and used to explore the pathogenesis of HD [33, 56, 86, 87]. In this study, we used N171-82Q TG mice and their WT littermates (control mice) to confirm previous reports that have shown that three identified SV2 isoforms (SV2A, B, and C) have different patterns of expression in the brain. While SV2A and SV2B have widespread distribution patterns, SV2C exhibits a more restricted pattern of expression in the brain [51, 52, 88, 89]. It is worth noting that in this study, the most prominent SV2C immunoreactivity was in the basal ganglia and hippocampus, both of which are known to be vulnerable in early-stage HD. Furthermore, the results of western blot analysis of whole brain lysates showed that while there was no significant difference in the protein levels of SV2A and SV2B, the SV2C protein was present at markedly lower levels in the N171-82Q TG mice than in the controls. Similar results were also obtained in an HD cell model. Thus, we chose to focus on SV2C in this study.

While Htt is widely distributed in the neurons of the brain, its mutations affect specific areas, and SV2C immunoreactivity was highly correlated with these areas. A reasonable explanation of this finding is that SV2C may be involved in the pathogenesis of HD. Because mutant Htt-induced synaptic dysfunctions and cognitive deficits have been attributed, in part, to the transcriptional dysregulation of presynaptic proteins involved in neurotransmitter release, such as complexin II, SNAP-25, and rabphilin 3a

[29, 64, 90–92], we assessed the expression of SV2C in the basal ganglia and hippocampus of an HD mouse model. We found that SV2C expression became progressively lower in the basal ganglia and hippocampus of HD TG mice than in WT controls.

To better understand the effect of mutant Htt on SV2C expression, we established an *in vitro* model using N2a cells, which have previously been used to model pathological features of HD [61, 62]. N2a cells possess some neuron-like characteristics [93] and endogenously express SV2C. They were therefore a good model system for this study. In agreement with our findings in an *in vivo* HD mouse model, we found that mutant Htt induced a significant decrease in the expression of SV2C at both the mRNA and protein level in the cell model. Our results support the hypothesis that transcriptional dysregulation is involved in HD pathogenesis [94–96]. Abnormal transcriptional alterations are known to be an early event in HD patients as well as HD cells and animal models [94]. Moreover, it has been suggested that mutant Htt impairs gene transcription by interacting with transcriptional regulatory proteins or other proteins that affect gene transcription or by inducing epigenetic changes in DNA [94, 97, 98]. Htt can interact with many transcription factors, including repressor element-1 silencing transcription factor (REST). REST is a transcriptional repressor that is sequestered in the cytoplasm when it forms a complex containing Htt [99]. Mutations in Htt disrupt this complex, leading to an increase in the translocation of REST into the nucleus and subsequent repression of the expression of its target genes [100, 101], which include SV2C (our unpublished data).

Although the exact physiological functions of SV2C remain unclear, emerging data indicate that SV2C plays an important role in the regulation of vesicle shape, trafficking, and exocytosis as well as dopamine activity [102–104], and its absence can lead to changes in basal

Fig. 5 Effect of mutant Htt on the expression of SV2C in an N2a cell model of HD. **A** Expression of the EGFP-Htt-120Q and EGFP-Htt-20Q fusion proteins in N2a cells at 48 h after transfection; arrows indicate aggregate formation (scale bar, 100 μm). **B** Western blot

analysis of SV2C protein expression levels at 24 h, 48 h and 72 h after transfection. **C** RT-PCR analysis of SV2C mRNA expression levels at 24 h, 48 h and 72 h after transfection. * $P < 0.05$, $n = 4$ /group.

ganglia-mediated behaviors or associative learning [102]. In addition, alterations in SV2C expression appear to be involved in the pathogenesis of Parkinson's disease (PD), epilepsy, and hippocampal sclerosis [84, 103, 105]. Furthermore, SV2C also plays a significant role in the pharmacology and toxicology of the nervous system. SV2C gene polymorphisms have been shown to impact responses to atypical antipsychotics, modulate the protective effect of nicotine on PD risk, and predict PD patient sensitivity to L-DOPA [106–108]. Moreover, SV2C has been shown to act as a receptor for botulinum neurotoxins,

which cause botulism and are used to treat some medical conditions and in cosmetic applications [109–111].

Here, we showed that SV2C is highly expressed in the basal ganglia and hippocampus of mice. Both of these regions are particularly vulnerable in HD and are involved in the pathogenesis underlying its early symptoms, which include cognitive deficits. The restricted distribution of SV2C could contribute to the region-selective pathology of HD. In the present study, we showed that there is a specific and progressive decrease in SV2C mRNA and protein expression in both HD mice and a cell model. These data

provide further evidence that transcriptional dysregulation and synaptic dysfunction may underlie the early symptoms and disease progression of HD. In addition, the results of our study support the notion that SV2C might be a useful therapeutic target for HD and a potential marker of HD progression.

Acknowledgements This work was supported by the National Natural Science Foundation of China (81371417).

Compliance with Ethical Standards

Conflict of Interest All authors claim that there are no conflicts of interest.

References

- MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, *et al.* A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993, 72: 971–983.
- Novak MJ, Tabrizi SJ. Huntington's disease. *BMJ* 2010, 340: c3109.
- Li HL, Zhang YB, Wu ZY. Development of research on Huntington disease in China. *Neurosci Bull* 2017, 33: 312–316.
- Du Z, Chazalon M, Bestaven E, Leste-Lasserre T, Baufreton J, Cazalets JR, *et al.* Early GABAergic transmission defects in the external globus pallidus and rest/activity rhythm alteration in a mouse model of Huntington's disease. *Neuroscience* 2016, 329: 363–379.
- Atherton JF, McIver EL, Mullen MR, Wokosin DL, Surmeier DJ, Bevan MD. Early dysfunction and progressive degeneration of the subthalamic nucleus in mouse models of Huntington's disease. *Elife* 2016, 5: e21616.
- Raymond LA, Andre VM, Cepeda C, Gladding CM, Milnerwood AJ, Levine MS. Pathophysiology of Huntington's disease: time-dependent alterations in synaptic and receptor function. *Neuroscience* 2011, 198: 252–273.
- Gos T, Krell D, Bielau H, Steiner J, Trubner K, Brisch R, *et al.* Demonstration of disturbed activity of external globus pallidus projecting neurons in depressed patients by the AgNOR staining method. *J Affect Disord* 2009, 119: 149–155.
- Hegeman DJ, Hong ES, Hernandez VM, Chan CS. The external globus pallidus: progress and perspectives. *Eur J Neurosci* 2016, 43: 1239–1265.
- Gittis AH, Berke JD, Bevan MD, Chan CS, Mallet N, Morrow MM, *et al.* New roles for the external globus pallidus in basal ganglia circuits and behavior. *J Neurosci* 2014, 34: 15178–15183.
- Jaeger D, Kita H. Functional connectivity and integrative properties of globus pallidus neurons. *Neuroscience* 2011, 198: 44–53.
- Gunaydin LA, Kreitzer AC. Cortico-basal ganglia circuit function in psychiatric disease. *Annu Rev Physiol* 2016, 78: 327–350.
- Sizemore RJ, Seeger-Armbruster S, Hughes SM, Parr-Brownlie LC. Viral vector-based tools advance knowledge of basal ganglia anatomy and physiology. *J Neurophysiol* 2016, 115: 2124–2146.
- Lehericy S, Bardin E, Poupon C, Vidailhet M, Francois C. 7 Tesla magnetic resonance imaging: a closer look at substantia nigra anatomy in Parkinson's disease. *Mov Disord* 2014, 29: 1574–1581.
- Glass M, Dragunow M, Faull RL. The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* 2000, 97: 505–519.
- Pancani T, Foster DJ, Moehle MS, Bichell TJ, Bradley E, Bridges TM, *et al.* Allosteric activation of M4 muscarinic receptors improve behavioral and physiological alterations in early symptomatic YAC128 mice. *Proc Natl Acad Sci U S A* 2015, 112: 14078–14083.
- Walker FO. Huntington's disease. *Lancet* 2007, 369: 218–228.
- Milnerwood AJ, Raymond LA. Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. *Trends Neurosci* 2010, 33: 513–523.
- Paulsen JS. Cognitive impairment in Huntington disease: diagnosis and treatment. *Curr Neurol Neurosci Rep* 2011, 11: 474–483.
- Morreale MK. Huntington's disease: looking beyond the movement disorder. *Adv Psychosom Med* 2015, 34: 135–142.
- Paulsen JS, Long JD. Onset of Huntington's disease: can it be purely cognitive? *Mov Disord* 2014, 29: 1342–1350.
- Martinen M, Kurkinen KM, Soininen H, Haapasalo A, Hiltunen M. Synaptic dysfunction and septin protein family members in neurodegenerative diseases. *Mol Neurodegener* 2015, 10: 16.
- Calabresi P, Picconi B, Tozzi A, Ghiglieri V. Interaction between basal ganglia and limbic circuits in learning and memory processes. *Parkinsonism Relat Disord* 2016, 22 Suppl 1: S65–68.
- Rubin RD, Watson PD, Duff MC, Cohen NJ. The role of the hippocampus in flexible cognition and social behavior. *Front Hum Neurosci* 2014, 8: 742.
- Cotterill RM. Cooperation of the basal ganglia, cerebellum, sensory cerebrum and hippocampus: possible implications for cognition, consciousness, intelligence and creativity. *Prog Neurobiol* 2001, 64: 1–33.
- Begeti F, Schwab LC, Mason SL, Barker RA. Hippocampal dysfunction defines disease onset in Huntington's disease. *J Neurol Neurosurg Psychiatry* 2016, 87: 975–981.
- Brito V, Gines S. p75NTR in Huntington's disease: beyond the basal ganglia. *Oncotarget* 2016, 7: 1–2.
- Brito V, Giral A, Enriquez-Barreto L, Puigdemelliv M, Suelves N, Zamora-Moratalla A, *et al.* Neurotrophin receptor p75(NTR) mediates Huntington's disease-associated synaptic and memory dysfunction. *J Clin Invest* 2014, 124: 4411–4428.
- Manczak M, Reddy PH. Mitochondrial division inhibitor 1 protects against mutant huntingtin-induced abnormal mitochondrial dynamics and neuronal damage in Huntington's disease. *Hum Mol Genet* 2015, 24: 7308–7325.
- Smith GA, Rocha EM, McLean JR, Hayes MA, Izen SC, Isacson O, *et al.* Progressive axonal transport and synaptic protein changes correlate with behavioral and neuropathological abnormalities in the heterozygous Q175 KI mouse model of Huntington's disease. *Hum Mol Genet* 2014, 23: 4510–4527.
- Rozas JL, Gomez-Sanchez L, Tomas-Zapico C, Lucas JJ, Fernandez-Chacon R. Presynaptic dysfunction in Huntington's disease. *Biochem Soc Trans* 2010, 38: 488–492.
- Nowack A, Yao J, Custer KL, Bajjalieh SM. SV2 regulates neurotransmitter release via multiple mechanisms. *Am J Physiol Cell Physiol* 2010, 299: C960–967.
- Shin OH. Exocytosis and synaptic vesicle function. *Compr Physiol* 2014, 4: 149–175.
- Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotz JA, *et al.* Intracellular inclusions and neuritic aggregates

- in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum Mol Genet* 1999, 8: 397–407.
34. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981, 29: 577–580.
 35. Liao M, Chen X, Han J, Yang S, Peng T, Li H. Selective expression of Huntingtin-associated protein 1 in β -cells of the rat pancreatic islets. *J Histochem Cytochem* 2010, 58: 255–263.
 36. Cregger M, Berger AJ, Rimm DL. Immunohistochemistry and quantitative analysis of protein expression. *Arch Pathol Lab Med* 2006, 130: 1026–1030.
 37. Wang CJ, Zhou ZG, Holmqvist A, Zhang H, Li Y, Adell G, *et al.* Survivin expression quantified by Image Pro-Plus compared with visual assessment. *Appl Immunohistochem Mol Morphol* 2009, 17: 530–535.
 38. Cha JH. Transcriptional dysregulation in Huntington's disease. *Trends Neurosci* 2000, 23: 387–392.
 39. Valor LM. Transcription, epigenetics and ameliorative strategies in Huntington's Disease: a genome-wide perspective. *Mol Neurobiol* 2015, 51: 406–423.
 40. van Hagen M, Piebes DGE, de Leeuw WC, Vuist IM, van Roon-Mom WMC, Moerland PD, *et al.* The dynamics of early-state transcriptional changes and aggregate formation in a Huntington's disease cell model. *BMC Genomics* 2017, 18: 373.
 41. Yu-Taeger L, Bonin M, Stricker-Shaver J, Riess O, Nguyen HH. Dysregulation of gene expression in the striatum of BACHD rats expressing full-length mutant huntingtin and associated abnormalities on molecular and protein levels. *Neuropharmacology* 2017, 117: 260–272.
 42. Bowles KR, Stone T, Holmans P, Allen ND, Dunnett SB, Jones L. SMAD transcription factors are altered in cell models of HD and regulate HTT expression. *Cell Signal* 2017, 31: 1–14.
 43. Lin L, Park JW, Ramachandran S, Zhang Y, Tseng YT, Shen S, *et al.* Transcriptome sequencing reveals aberrant alternative splicing in Huntington's disease. *Hum Mol Genet* 2016, 25: 3454–3466.
 44. Langfelder P, Cante JP, Chatzopoulou D, Wang N, Gao F, Al-Ramahi I, *et al.* Integrated genomics and proteomics define huntingtin CAG length-dependent networks in mice. *Nat Neurosci* 2016, 19: 623–633.
 45. Moumne L, Betuing S, Caboche J. Multiple aspects of gene dysregulation in Huntington's disease. *Front Neurol* 2013, 4: 127.
 46. Potter MC, Yuan C, Ottenritter C, Mughal M, van Praag H. Exercise is not beneficial and may accelerate symptom onset in a mouse model of Huntington's disease. *PLoS Curr* 2010, 2: RRN1201.
 47. Cheng Y, Peng Q, Hou Z, Aggarwal M, Zhang J, Mori S, *et al.* Structural MRI detects progressive regional brain atrophy and neuroprotective effects in N171-82Q Huntington's disease mouse model. *Neuroimage* 2011, 56: 1027–1034.
 48. Wang CE, Tydlacka S, Orr AL, Yang SH, Graham RK, Hayden MR, *et al.* Accumulation of N-terminal mutant huntingtin in mouse and monkey models implicated as a pathogenic mechanism in Huntington's disease. *Hum Mol Genet* 2008, 17: 2738–2751.
 49. Jin J, Cheng Y, Zhang Y, Wood W, Peng Q, Hutchison E, *et al.* Interrogation of brain miRNA and mRNA expression profiles reveals a molecular regulatory network that is perturbed by mutant huntingtin. *J Neurochem* 2012, 123: 477–490.
 50. Ferrante RJ. Mouse models of Huntington's disease and methodological considerations for therapeutic trials. *Biochim Biophys Acta* 2009, 1792: 506–520.
 51. Janz R, Sudhof TC. SV2C is a synaptic vesicle protein with an unusually restricted localization: anatomy of a synaptic vesicle protein family. *Neuroscience* 1999, 94: 1279–1290.
 52. Dardou D, Dassel D, Cuvelier L, Deprez T, De Ryck M, Schiffmann SN. Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. *Brain Res* 2011, 1367: 130–145.
 53. Dunn AR, Hoffman CA, Stout KA, Ozawa M, Dhamsania RK, Miller GW. Immunohistochemical analysis of the expression of SV2C in mouse, macaque and human brain. *Brain Res* 2017. <https://doi.org/10.1016/j.brainres.2017.12.029>.
 54. McBride JL, Ramaswamy S, Gasmi M, Bartus RT, Herzog CD, Brandon EP, *et al.* Viral delivery of glial cell line-derived neurotrophic factor improves behavior and protects striatal neurons in a mouse model of Huntington's disease. *Proc Natl Acad Sci U S A* 2006, 103: 9345–9350.
 55. Kolodziejczyk K, Parsons MP, Southwell AL, Hayden MR, Raymond LA. Striatal synaptic dysfunction and hippocampal plasticity deficits in the Hu97/18 mouse model of Huntington disease. *PLoS One* 2014, 9: e94562.
 56. Yu ZX, Li SH, Evans J, Pillarisetti A, Li H, Li XJ. Mutant huntingtin causes context-dependent neurodegeneration in mice with Huntington's disease. *J Neurosci* 2003, 23: 2193–2202.
 57. Ramaswamy S, McBride JL, Kordower JH. Animal models of Huntington's disease. *ILAR J* 2007, 48: 356–373.
 58. Heng MY, Detloff PJ, Albin RL. Rodent genetic models of Huntington disease. *Neurobiol Dis* 2008, 32: 1–9.
 59. Maier T, Guell M, Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett* 2009, 583: 3966–3973.
 60. Koussounadis A, Langdon SP, Um IH, Harrison DJ, Smith VA. Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci Rep* 2015, 5: 10775.
 61. Cisbani G, Cicchetti F. An *in vitro* perspective on the molecular mechanisms underlying mutant huntingtin protein toxicity. *Cell Death Dis* 2012, 3: e382.
 62. Schlachetzki JC, Saliba SW, Oliveira AC. Studying neurodegenerative diseases in culture models. *Rev Bras Psiquiatr* 2013, 35 Suppl 2: S92–100.
 63. Reiner A, Dragatsis I, Dietrich P. Genetics and neuropathology of Huntington's disease. *Int Rev Neurobiol* 2011, 98: 325–372.
 64. Smith R, Brundin P, Li JY. Synaptic dysfunction in Huntington's disease: a new perspective. *Cell Mol Life Sci* 2005, 62: 1901–1912.
 65. Li JY, Plomann M, Brundin P. Huntington's disease: a synaptopathy? *Trends Mol Med* 2003, 9: 414–420.
 66. Sepers MD, Raymond LA. Mechanisms of synaptic dysfunction and excitotoxicity in Huntington's disease. *Drug Discov Today* 2014, 19: 990–996.
 67. Murmu RP, Li W, Szepesi Z, Li JY. Altered sensory experience exacerbates stable dendritic spine and synapse loss in a mouse model of Huntington's disease. *J Neurosci* 2015, 35: 287–298.
 68. Puigdelivol M, Saavedra A, Perez-Navarro E. Cognitive dysfunction in Huntington's disease: mechanisms and therapeutic strategies beyond BDNF. *Brain Pathol* 2016, 26: 752–771.
 69. Raymond LA. Striatal synaptic dysfunction and altered calcium regulation in Huntington disease. *Biochem Biophys Res Commun* 2017, 483: 1051–1062.
 70. Nithianantharajah J, Hannan AJ. Dysregulation of synaptic proteins, dendritic spine abnormalities and pathological plasticity of synapses as experience-dependent mediators of cognitive and psychiatric symptoms in Huntington's disease. *Neuroscience* 2013, 251: 66–74.

71. Tyebji S, Hannan AJ. Synaptopathic mechanisms of neurodegeneration and dementia: Insights from Huntington's disease. *Prog Neurobiol* 2017, 153: 18–45.
72. Silva FR, Miranda AS, Santos RPM, Olmo IG, Zamponi GW, Dobransky T, *et al.* N-type Ca(2+) channels are affected by full-length mutant huntingtin expression in a mouse model of Huntington's disease. *Neurobiol Aging* 2017, 55: 1–10.
73. Hachigian LJ, Carmona V, Fenster RJ, Kulicke R, Heilbut A, Sittler A, *et al.* Control of Huntington's disease-associated phenotypes by the striatum-enriched transcription factor Foxp2. *Cell Rep* 2017, 21: 2688–2695.
74. Montoya A, Price BH, Menear M, Lepage M. Brain imaging and cognitive dysfunctions in Huntington's disease. *J Psychiatry Neurosci* 2006, 31: 21–29.
75. Buren C, Parsons MP, Smith-Dijk A, Raymond LA. Impaired development of cortico-striatal synaptic connectivity in a cell culture model of Huntington's disease. *Neurobiol Dis* 2016, 87: 80–90.
76. Wolf RC, Sambataro F, Vasic N, Schonfeldt-Lecuona C, Ecker D, Landwehrmeyer B. Altered frontostriatal coupling in pre-manifest Huntington's disease: effects of increasing cognitive load. *Eur J Neurol* 2008, 15: 1180–1190.
77. Lawrence AD, Hodges JR, Rosser AE, Kershaw A, Ffrench-Constant C, Rubinsztein DC, *et al.* Evidence for specific cognitive deficits in preclinical Huntington's disease. *Brain* 1998, 121 (Pt 7): 1329–1341.
78. Rangel-Barajas C, Rebec GV. Dysregulation of corticostriatal connectivity in Huntington's disease: a role for dopamine modulation. *J Huntingtons Dis* 2016, 5: 303–331.
79. Wikenheiser AM, Schoenbaum G. Over the river, through the woods: cognitive maps in the hippocampus and orbitofrontal cortex. *Nat Rev Neurosci* 2016, 17: 513–523.
80. Anglada-Huguet M, Vidal-Sancho L, Giralto A, Garcia-Diaz Barriga G, Xifro X, Alberch J. Prostaglandin E2 EP2 activation reduces memory decline in R6/1 mouse model of Huntington's disease by the induction of BDNF-dependent synaptic plasticity. *Neurobiol Dis* 2016, 95: 22–34.
81. Miguez A, Garcia-Diaz Barriga G, Brito V, Straccia M, Giralto A, Gines S, *et al.* Fingolimod (FTY720) enhances hippocampal synaptic plasticity and memory in Huntington's disease by preventing p75NTR up-regulation and astrocyte-mediated inflammation. *Hum Mol Genet* 2015, 24: 4958–4970.
82. Ghilan M, Bostrom CA, Hryciw BN, Simpson JM, Christie BR, Gil-Mohapel J. YAC128 Huntington's disease transgenic mice show enhanced short-term hippocampal synaptic plasticity early in the course of the disease. *Brain Res* 2014, 1581: 117–128.
83. Dallerac GM, Cummings DM, Hirst MC, Milnerwood AJ, Murphy KP. Changes in dopamine signalling do not underlie aberrant hippocampal plasticity in a mouse model of Huntington's disease. *Neuromolecular Med* 2016, 18: 146–153.
84. Loscher W, Gillard M, Sands ZA, Kaminski RM, Klitgaard H. Synaptic vesicle glycoprotein 2a ligands in the treatment of epilepsy and beyond. *CNS Drugs* 2016, 30: 1055–1077.
85. Wan QF, Zhou ZY, Thakur P, Vila A, Sherry DM, Janz R, *et al.* SV2 acts via presynaptic calcium to regulate neurotransmitter release. *Neuron* 2010, 66: 884–895.
86. Jia H, Wang Y, Morris CD, Jacques V, Gottesfeld JM, Rusche JR, *et al.* The effects of pharmacological inhibition of histone deacetylase 3 (HDAC3) in Huntington's disease mice. *PLoS One* 2016, 11: e0152498.
87. Damiano M, Digué E, Maltorn C, D'Aurelio M, Galvan L, Petit F, *et al.* A role of mitochondrial complex II defects in genetic models of Huntington's disease expressing N-terminal fragments of mutant huntingtin. *Hum Mol Genet* 2013, 22: 3869–3882.
88. Bajjalieh SM, Frantz GD, Weimann JM, McConnell SK, Scheller RH. Differential expression of synaptic vesicle protein 2 (SV2) isoforms. *J Neurosci* 1994, 14: 5223–5235.
89. Crevecoeur J, Foerch P, Doupagne M, Thielen C, Vandenplas C, Moonen G, *et al.* Expression of SV2 isoforms during rodent brain development. *BMC Neurosci* 2013, 14: 87.
90. Morton AJ, Edwardson JM. Progressive depletion of complexin II in a transgenic mouse model of Huntington's disease. *J Neurochem* 2001, 76: 166–172.
91. Morton AJ, Faull RL, Edwardson JM. Abnormalities in the synaptic vesicle fusion machinery in Huntington's disease. *Brain Res Bull* 2001, 56: 111–117.
92. Smith R, Petersen A, Bates GP, Brundin P, Li JY. Depletion of rabphilin 3A in a transgenic mouse model (R6/1) of Huntington's disease, a possible culprit in synaptic dysfunction. *Neurobiol Dis* 2005, 20: 673–684.
93. Poirier MA, Jiang H, Ross CA. A structure-based analysis of huntingtin mutant polyglutamine aggregation and toxicity: evidence for a compact beta-sheet structure. *Hum Mol Genet* 2005, 14: 765–774.
94. Glajch KE, Sadri-Vakili G. Epigenetic mechanisms involved in Huntington's disease pathogenesis. *J Huntingtons Dis* 2015, 4: 1–15.
95. Kumar A, Vaish M, Ratan RR. Transcriptional dysregulation in Huntington's disease: a failure of adaptive transcriptional homeostasis. *Drug Discov Today* 2014, 19: 956–962.
96. Kazantsev AG, Hersch SM. Drug targeting of dysregulated transcription in Huntington's disease. *Prog Neurobiol* 2007, 83: 249–259.
97. Sari Y. Huntington's disease: from mutant Huntingtin protein to neurotrophic factor therapy. *Int J Biomed Sci* 2011, 7: 89–100.
98. Sugars KL, Rubinsztein DC. Transcriptional abnormalities in Huntington disease. *Trends Genet* 2003, 19: 233–238.
99. Shimojo M. Huntingtin regulates RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) nuclear trafficking indirectly through a complex with REST/NRSF-interacting LIM domain protein (RILP) and dynactin p150 Glued. *J Biol Chem* 2008, 283: 34880–34886.
100. Soldati C, Bithell A, Johnston C, Wong KY, Stanton LW, Buckley NJ. Dysregulation of REST-regulated coding and non-coding RNAs in a cellular model of Huntington's disease. *J Neurochem* 2013, 124: 418–430.
101. Zuccato C, Belyaev N, Conforti P, Ooi L, Tartari M, Papadimou E, *et al.* Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. *J Neurosci* 2007, 27: 6972–6983.
102. Dardou D, Monlezun S, Foerch P, Courade JP, Cuvelier L, De Ryck M, *et al.* A role for Sv2c in basal ganglia functions. *Brain Res* 2013, 1507: 61–73.
103. Dunn AR, Stout KA, Ozawa M, Lohr KM, Hoffman CA, Bernstein AI, *et al.* Synaptic vesicle glycoprotein 2C (SV2C) modulates dopamine release and is disrupted in Parkinson disease. *Proc Natl Acad Sci U S A* 2017, 114: E2253–E2262.
104. Schivell AE, Mochida S, Kensel-Hammes P, Custer KL, Bajjalieh SM. SV2A and SV2C contain a unique synaptotagmin-binding site. *Mol Cell Neurosci* 2005, 29: 56–64.
105. Crevecoeur J, Kaminski RM, Rogister B, Foerch P, Vandenplas C, Neveux M, *et al.* Expression pattern of synaptic vesicle protein 2 (SV2) isoforms in patients with temporal lobe epilepsy and hippocampal sclerosis. *Neuropathol Appl Neurobiol* 2014, 40: 191–204.
106. Altmann V, Schumacher-Schuh AF, Rieck M, Callegari-Jacques SM, Rieder CR, Hutz MH. Influence of genetic, biological and pharmacological factors on levodopa dose in Parkinson's disease. *Pharmacogenomics* 2016, 17: 481–488.

107. Hill-Burns EM, Singh N, Ganguly P, Hamza TH, Montimurro J, Kay DM, *et al.* A genetic basis for the variable effect of smoking/nicotine on Parkinson's disease. *Pharmacogenomics J* 2013, 13: 530–537.
108. Ramsey TL, Liu Q, Massey BW, Brennan MD. Genotypic variation in the SV2C gene impacts response to atypical antipsychotics the CATIE study. *Schizophr Res* 2013, 149: 21–25.
109. Wang F, Wan H, Hu JP, Chang S. Molecular dynamics simulations of wild type and mutants of botulinum neurotoxin A complexed with synaptic vesicle protein 2C. *Mol Biosyst* 2015, 11: 223–231.
110. Strotmeier J, Mahrhold S, Krez N, Janzen C, Lou J, Marks JD, *et al.* Identification of the synaptic vesicle glycoprotein 2 receptor binding site in botulinum neurotoxin A. *FEBS Lett* 2014, 588: 1087–1093.
111. Benoit RM, Frey D, Hilbert M, Kevenaar JT, Wieser MM, Stirnimann CU, *et al.* Structural basis for recognition of synaptic vesicle protein 2C by botulinum neurotoxin A. *Nature* 2014, 505: 108–111.