

Case report

Divalent cation-responsive myotonia and muscle paralysis in skeletal muscle sodium channelopathy

Ami Mankodi ^{a,*}, Christopher Grunseich ^a, Martin Skov ^b, Lisa Cook ^c, Georg Aue ^c,
Enkhtsetseg Purev ^c, Dara Bakar ^a, Tanya Lehky ^d, Karin Jurkat-Rott ^e, Thomas H. Pedersen ^b,
Richard W. Childs ^c

^a Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA

^b Department of Biomedicine, University of Aarhus, 8000 Aarhus, Denmark

^c Section of Transplantation Immunotherapy, National Heart, Lung, and Blood Institute, Bethesda, MD 20892, USA

^d Clinical Neurophysiology Program, EMG Section, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA

^e Division of Neurophysiology, University of Ulm, 89069 Ulm, Germany

Received 8 July 2015; received in revised form 11 August 2015; accepted 14 August 2015

Abstract

We report a patient with paramyotonia congenita/hyperkalemic periodic paralysis due to Nav1.4 I693T mutation who had worsening of myotonia and muscle weakness in the setting of hypomagnesemia and hypocalcemia with marked recovery after magnesium administration. Computer simulations of the effects of the I693T mutation were introduced in the muscle fiber model by both hyperpolarizing shifts in the Nav1.4 channel activation and a faster recovery from slow channel inactivation. A further shift in the Nav1.4 channel activation in the hyperpolarizing direction as expected with low divalent cations resulted in myotonia that progressed to membrane inexcitability. Shifting the channel activation in the depolarizing direction as would be anticipated from magnesium supplementation abolished the myotonia. These observations provide clinical and biophysical evidence that the muscle symptoms in sodium channelopathy are sensitive to divalent cations. Exploration of the role of magnesium administration in therapy or prophylaxis is warranted with a randomized clinical trial.

Published by Elsevier B.V.

Keywords: Myotonia; Paramyotonia congenita; Periodic paralysis; Muscle weakness; Sodium channel; Magnesium

1. Introduction

Skeletal muscle sodium channelopathy and childhood-onset aplastic anemia are rare. Concurrence of these rare diseases in the same subject has not previously been reported. Myotonia and muscle weakness are cardinal symptoms in sodium channel disorders of the skeletal muscle. Experimental studies have shown that reduced concentrations of extracellular Mg^{2+} and Ca^{2+} ions exacerbate myotonia in the CLC-1 chloride channel inhibited skeletal muscle fibers [1,2]. Effects of serum divalent cation concentrations on myotonia and muscle weakness in patients are not yet known.

We present a patient with paramyotonia congenita/hyperkalemic periodic paralysis and aplastic anemia who had

worsening myotonia and prolonged muscle paralytic episodes in the setting of drug-induced hypomagnesemia and hypocalcemia. His symptoms improved with the normalization of the serum divalent cation concentration. We provide supporting biophysical data using a mathematical simulation model of single skeletal muscle fibers. The Institutional Review Board approved the study and the patient provided written informed consent for the contribution to this study.

2. Case report

A 19-year-old man reported episodes of muscle stiffness followed by weakness that lasted ≤ 30 min since the age of 3 years. Muscles in the face, arms and legs were affected. Episodes occurred about once a week and did not interfere with his quality of life. The episodes were triggered by rest after exercise, prolonged sleep, and hunger. His symptoms improved during the teenage years such that he only noticed transient right upper arm stiffness about 2–3 times in a month. A similar history in his father suggested dominant inheritance.

* Corresponding author. Neurogenetics Branch, NINDS/NIH, 35 Convent Drive, Building 35, Room 2A-1002, Bethesda, MD 20892-3075, USA. Tel.: +1 301 827 6690; fax: +1 301 480 3365.

E-mail address: Ami.Mankodi@nih.gov (A. Mankodi).

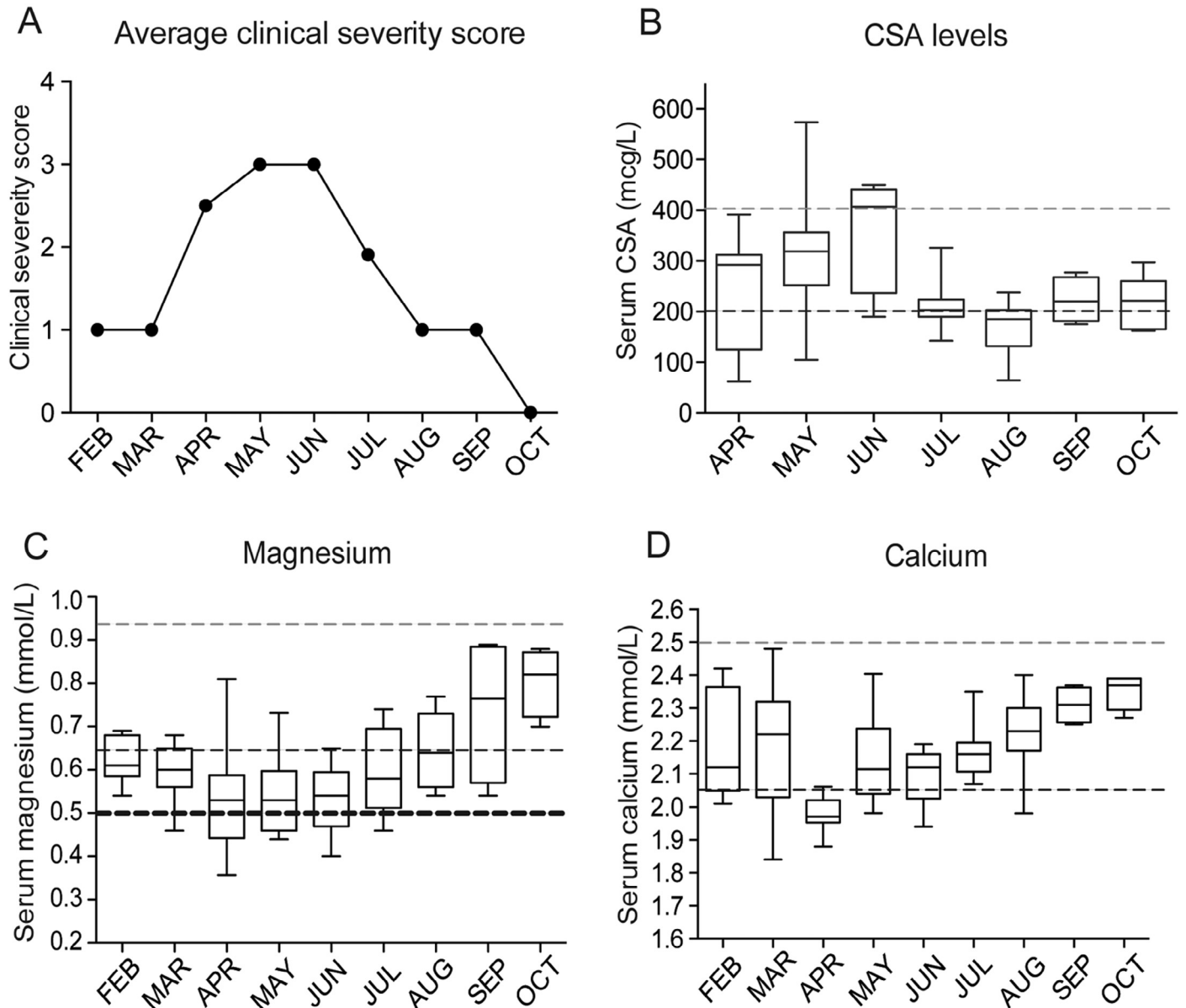


Fig. 1. Clinical and laboratory findings over time (A–D). Clinical severity score (A) was adapted from the GBS disability scoring system (0: A healthy state, 1: Minor symptoms and capable of running, 2: Needs help with walking, 3: Chair bound or bedridden). Average monthly CSA (B), Mg^{2+} (C) and Ca^{2+} (D) concentrations. Monthly data are shown in box and whiskers format with 5% and 95% confidence intervals. Light and dark lines indicate the normal laboratory reference range for divalent cations and therapeutic range for CSA. Heavy dark line (C) defines a critically low magnesium level.

He was treated with multiple courses of immunosuppressive therapy (IST) including cyclosporine (CSA) for aplastic anemia from the age of 9 years. Due to worsening aplastic anemia, he received an allogeneic hematopoietic stem cell transplant using haploidentical CD34+ cells from his mother combined with a single umbilical cord blood unit at the age of 20 years. In the weeks following the transplant he developed daily symptoms of stiffness followed by generalized flaccid paralysis lasting for >8 hours (Fig. 1A). Bulbar, respiratory and sphincter muscles were not affected. Previously reported triggers such as rest after exercise, cold environment, hunger and excess sleep were absent. In between the episodes, his muscle strength was normal. Eyelid myotonia was present and worsened on repetitive

exercise. Repetitive forceful handgrip exercise induced myotonia. Cooling of the forearms resulted in muscle weakness. Thyroid hormone levels were normal. Electromyography showed myotonia and myopathic changes in the limb muscles. DNA testing confirmed the presence of a previously characterized p.I693T mutation in the domain II S4-S5 linker of the alpha subunit of the voltage gated skeletal muscle sodium channel (Nav1.4) [3,4]. Post-transplant IST consisted of CSA given at doses to maintain levels in the therapeutic range (200–400 mcg/L) (Fig. 1B) and mycophenolate mofetil 1000 mg twice daily. Although serum K^+ levels remained within physiological range, serum Mg^{2+} and Ca^{2+} levels dropped below 0.5 mmol/L and 2.2 mmol/L, respectively (Fig. 1C and D). Mg^{2+}

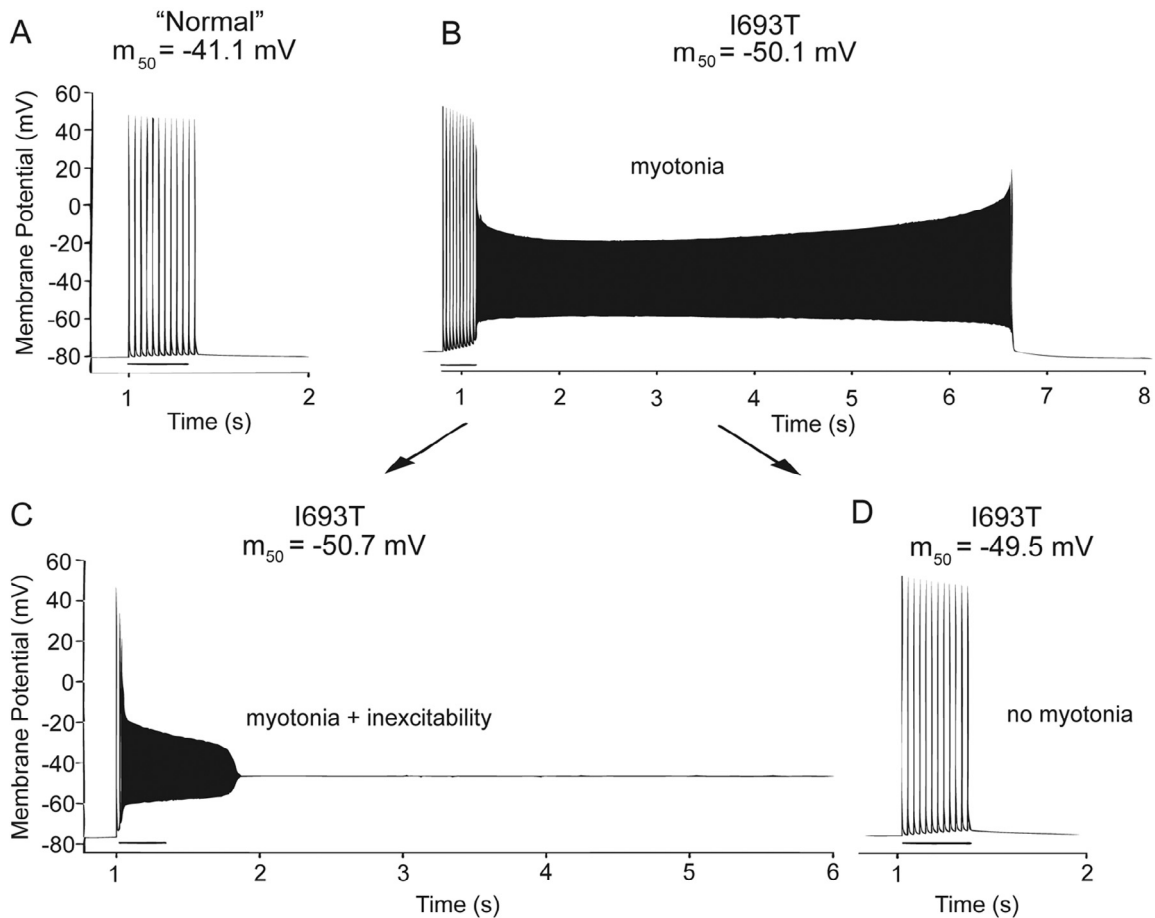


Fig. 2. Computerized model of action potential firing in single muscle fibers (A–D). Voltage gated ion channels in the model were described using Hodgkin–Huxley kinetics [5]. Panels show membrane potential from a fiber stimulated at 30 Hz for 400 ms. (A) shows normal firing of action potentials in a fiber with activation curve midpoint (m_{50}) = -41.1 mV; representing the “normal state”. (B) shows a burst of myotonia lasting over 6 seconds in a fiber with m_{50} shifted by 9 mV in a hyperpolarizing direction from -41.1 to -50.1 mV along with a 5-fold increased rate of recovery from slow inactivation; representing the “Nav1.4 I693T” effects. (C and D) show results of m_{50} shifts in fibers with the “Nav1.4 I693T” effects. (C) shows myotonia precipitating in an inexcitable state in a fiber with m_{50} shifted by 0.6 mV in the hyperpolarizing direction from -50.1 mV to -50.7 mV; representing subnormal levels of divalent cations. (D) shows return to a normal excitability state in a fiber with m_{50} shifted by 0.6 mV in the depolarizing direction from -50.1 mV to -49.5 mV; representing response to magnesium administration.

supplements, both intravenous and oral, were given to correct the hypomagnesemia. Hypocalcemia was resolved with magnesium supplementation. He did not receive simultaneous calcium supplements. He had daily episodes of stiffness followed by prolonged muscle paralysis until serum Mg^{2+} and Ca^{2+} levels were in the normal reference range (Fig. 1A, C, D). He continued taking Mg^{2+} supplement 1200 mg PO TID. At the time of this report he has remained symptom-free for more than 18 months following correction of hypomagnesemia and hypocalcemia. Since he has had no signs of graft versus host disease, we plan to taper him off CSA over the next 6–12 months.

2.1. The role of divalent cations in alleviating symptoms in skeletal muscle sodium channelopathy due to Nav1.4 I693T mutation

Previous studies showed that reductions in extracellular Mg^{2+} and Ca^{2+} exacerbate myotonia induced by CIC-1 chloride channel inhibition in isolated human [2] and rat [1] muscle. The close association between correction of hypomagnesemia and hypocalcemia, and the resolution of muscle symptoms in this

patient strongly suggests that low serum Mg^{2+} and Ca^{2+} also exacerbate muscle symptoms in sodium channelopathy. A mathematical model of single muscle fibers [1,5] was used to better understand the effects of divalent cations on the voltage dependence of Nav1.4 I693T channel activation (Fig. 2A–D). The I693T mutation causes a 9 mV hyperpolarizing shift in Nav1.4 activation [3], along with a faster recovery of Nav1.4 from slow inactivation [6]. In the modeled muscle fibers, the I693T effects were reproduced by shifting the Nav1.4 activation parameter (m_{50}) in the hyperpolarized direction from -41.1 to -50.1 mV and simultaneously increasing the off kinetics of the slow inactivation by 5-fold [7]. These Nav1.4 alterations in the model resulted in brief trains of stimulated action potentials precipitating into myotonia that lasted for a few seconds (Fig. 2B). Increased extracellular Mg^{2+} and Ca^{2+} ions have been shown to cause a shift in the Nav1.4 activation in the depolarizing direction, thereby effectively raising the action potential threshold [8–11]. Subnormal levels of divalent cations would be expected to exacerbate the genetically induced hyperpolarizing shift in the Nav1.4 activation in the patient. In

the modeled muscle fibers a leftward hyperpolarizing shift by only 0.6 mV in the Nav1.4 activation (−50.1 to −50.7 mV) induced myotonia followed by a prolonged depolarization state during which the muscle membrane became inexcitable reminiscent of muscle paralysis (Fig. 2C). Conversely, a depolarizing shift in the Nav1.4 activation by 0.6 mV (−50.1 to −49.5), as would be expected from increasing serum Mg^{2+} and Ca^{2+} , abolished myotonic discharges in the modeled muscle fibers (Fig. 2D). In the clinical setting, shifting of Nav1.4 activation by divalent cations is limited by tight regulation of the serum divalent cation concentration within a narrow range. Accordingly, our simulations showed that very small changes in the Nav1.4 activation parameter (m_{50}) resulted in marked changes in susceptibility to myotonia and loss of membrane excitability.

3. Discussion

We describe a patient with an autosomal dominant sodium channelopathy due to Nav1.4 I693T mutation who had an excellent recovery from muscle symptoms with Mg^{2+} supplementation. Clinical presentation of brief episodes of focal stiffness followed by weakness involving the face and upper body, relatively non-progressive disease course and myotonia induced and exacerbated by cold and repetitive exercise indicated a diagnosis of paramyotonia congenita. Prolonged episodes of generalized flaccid muscle paralysis lasting for several hours suggested a phenotype overlap with hyperkalemic periodic paralysis. This agrees well with the fact that the Nav1.4 I693T mutation has been previously reported in patients with paramyotonia congenita [3,4] as well as hyperkalemic periodic paralysis [12].

The symptoms of episodic muscle weakness and myotonia were aggravated in the setting of hypomagnesemia and hypocalcemia. Deficiency of divalent cations is commonly associated with neuromuscular hyperexcitability symptoms including tremor, fasciculations, and tetany. In contrast, the patient developed prolonged episodes of muscle paralysis such that he was bedridden or chair bound. The abnormalities in divalent cations were ascribed to CSA-induced renal Mg^{2+} wasting as described in the literature [13]. Our patient improved with the correction of serum divalent cations while still continuing CSA. Increased Ca^{2+} sensitivity manifested by transient weakness when the Ca^{2+} levels were lowered to 0.5 mM and faster recovery of muscle function following repletion of Ca^{2+} has been demonstrated in a knock-in mouse model of the Nav1.4 M1592V mutation [14]. However, the mechanism by which Ca^{2+} modulates susceptibility to muscle paralysis is not yet understood in the mouse model.

The physiological basis of divalent cation effect on muscle membrane excitability is better understood for myotonia than for muscle weakness. Previous studies showed that lowering of extracellular divalent cations aggravated myotonic discharges and increasing divalent cations reduced myotonia in CIC-1 inhibited muscle fibers [1,2]. This reduction in myotonia by divalent cations was explained by a depolarizing shift in the membrane potential required to activate Nav1.4, effectively

increasing the action potential threshold. Our report provides clinical evidence that variation in serum divalent cation concentration can impact symptoms of myotonia and muscle weakness in patients with sodium channelopathy. However, the cellular mechanism underlying myotonia differs markedly between chloride and sodium channelopathies, and it is not known whether a depolarizing shift in Nav1.4 activation could also reduce myotonia caused by an abnormal Nav1.4 function. To address this question we used mathematical simulations of action potential firing in single muscle fibers. In the model, myotonia was induced by reproducing the effects of the I693T mutation on Nav1.4 channel function. In this state the model became very sensitive to small shifts in Nav1.4 activation such that only the 0.6 mV depolarizing shift was sufficient to abolish myotonia. Moreover, when the Nav1.4 activation was shifted by 0.6 mV in a hyperpolarizing direction, as would be expected to occur with lowering of Mg^{2+} and Ca^{2+} , the muscle fiber became inexcitable after firing a few action potentials. This is highly compatible with muscle paralysis that was observed in the patient in the state of drug-induced divalent cation deficiency.

The present study provides clinical evidence and support from mathematical modeling that muscle symptoms with sodium channel mutations are sensitive to serum divalent cation concentrations. Together with previous in vitro studies of CIC-1 inhibited muscle [1,2], it appears that the muscle symptoms can be improved when extracellular divalent cation concentrations are maintained at the upper end of their physiological range. These findings point to further investigation for Mg^{2+} supplementation as a prophylaxis or therapy in patients with chloride or sodium channelopathy with a randomized clinical trial.

Acknowledgements

We thank our patient and his family for their contribution. We thank Dr. Kenneth H. Fischbeck for critically reading the manuscript. This work was supported by intramural funding from the National Institutes of Health, Novo Nordisk Foundation (THP) (Grant No. NNF14OC0013143) and the Hertie Foundation, the German Research Ministry (IonNeurONet) and the German Muscle Disease Society DGM (KJR).

References

- [1] Skov M, Riisager A, Fraser JA, et al. Extracellular magnesium and calcium reduce myotonia in CIC-1 inhibited rat muscle. *Neuromuscul Disord* 2013;23:489–502.
- [2] Skov M, de Paoli FV, Lausten J, et al. Extracellular magnesium and calcium reduce myotonia in isolated CIC-1 inhibited human muscle. *Muscle Nerve* 2015;51:65–71.
- [3] Plassart-Schiess E, Lhuillier L, George AL Jr, et al. Functional expression of the Ile693Thr Na^+ channel mutation associated with paramyotonia congenita in a human cell line. *J Physiol* 1998;507:721–7.
- [4] Plassart E, Eymard B, Maurs L, et al. Paramyotonia congenita: genotype to phenotype correlations in two families and report of a new mutation in the sodium channel gene. *J Neurol Sci* 1996;142:126–33.
- [5] Fraser JA, Huang CL, Pedersen TH. Relationships between resting conductances, excitability, and t-system ionic homeostasis in skeletal muscle. *J Gen Physiol* 2011;138:95–116.

- [6] Hayward LJ, Sandowal GM, Cannon SC. Defective slow inactivation of sodium channels contributes to familial periodic paralysis. *Neurology* 1999;52:1447–53.
- [7] Karoly R, Lenkey N, Juhasz AO, Vizi ES, Mike A. Fast- or slow-inactivated state preference of Na⁺ channel inhibitors: a simulation and experimental study. *PLoS Comput Biol* 2010;6:e1000818.
- [8] Hille B, Woodhull AM, Shapiro BI. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Philos Trans R Soc Lond B Biol Sci* 1975;270:301–18.
- [9] Green WN, Andersen OS. Surface charges and ion channel function. *Annu Rev Physiol* 1991;53:341–59.
- [10] Cukierman S, Krueger BK. Effects of internal divalent cations on the gating of rat brain Na⁺ channels reconstituted in planar lipid bilayers. *Pflugers Arch* 1991;419:559–65.
- [11] Cukierman S, Krueger BK. Modulation of sodium channel gating by external divalent cations: differential effects on opening and closing rates. *Pflugers Arch* 1990;416:360–7.
- [12] Jurkat-Rott K, Lehmann-Horn F. Genotype-phenotype correlation and therapeutic rationale in hyperkalemic periodic paralysis. *Neurother* 2007;4:216–24.
- [13] Ledeganck KJ, De Winter BY, Van den Driessche A, et al. Magnesium loss in cyclosporine-treated patients is related to renal epidermal growth factor downregulation. *Nephrol Dial Transplant* 2014;29:1097–102.
- [14] Hayward LJ, Kim JS, Lee MY, et al. Targeted mutation of mouse skeletal muscle sodium channel produces myotonia and potassium-sensitive weakness. *J Clin Invest* 2008;118:1437–49.