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Altered expression of potassium channel genes in familial hypokalemic periodic paralysis

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Abstract

We analyzed the mRNA expression patterns of major potassium channel genes to determine the mechanism of hypokalemia in familial hypokalemic periodic paralysis. We used quantitative RT-PCR to examine the mRNA levels of both inward (*KCNJ2*, *KCNJ6*, and *KCNJ14*) and delayed rectifier (*KCNQ1* and *KCNA2*) potassium channel genes in skeletal muscle cells from both normal and patient groups, prior to and after exposure to 4 mM and 50 mM potassium buffers. Quantitative RT-PCR analysis revealed no changes in the mRNA levels of these genes in normal and patient cells on exposure to 4 mM potassium buffer. However, after exposure to 50 mM potassium buffer, which was used to induce depolarization, normal cells showed a significant decrease in *KCNJ2*, *KCNJ6*, and *KCNJ14* expression, but no change in *KCNQ1* and *KCNA2* expression. In contrast, patient cells showed no change in *KCNJ2* and *KCNJ6* expression, but an increase in *KCNJ14* expression. Furthermore, *KCNQ1* and *KCNA2* showed decreased expression. We found that the expression levels of both inward and delayed rectifier potassium channel genes in patient cells differ from those in normal cells. Altered potassium channel gene expression in patient cells may suggest a possible mechanism for hypokalemia in familial hypokalemic periodic paralysis.

INTRODUCTION

Familial hypokalemic periodic paralysis (OMIM ID: 170400) is an autosomal-dominant channelopathy that is characterized by episodic attacks of flaccid paralysis and concomitant hypokalemia. The age of the first attack varies from infancy to puberty, with most cases occurring around puberty. The attacks commonly occur at dawn or in the early morning and can last for hours and sometimes for days. Excessive ingestion of sweet or salty foods, strenuous exercise, or exposure to cold or emotional stress can trigger paralytic attacks. Molecular genetic studies have shown that the disease is caused by mutations in the calcium channel gene (*CACNA1S*) or the sodium channel gene (*SCN4A*), but the mechanism that causes hypokalemia remains undetermined.[1-3]

Inward rectifier potassium channels (Kir) play central roles in regulating membrane potential. There are seven subfamilies of Kir channels, denoted as Kir1 to Kir7. Abnormal function of the channels has been associated with human diseases.[4-6] *KCNJ2* is the Kir2.1-encoding gene. Kir2.1 functions in the transfer of electric signals by transporting intracellular or extracellular potassium ions. It plays a crucial role in skeletal muscle contraction and relaxation.[7] *KCNJ6* is the Kir3.2-encoding gene and is under the control of a G-protein. Increased concentration of adenosine triphosphate in pancreatic cells results in the closure of Kir3.2, triggering depolarization and the secretion of insulin outside the cells.[8] *KCNJ14* is the Kir2.4-encoding gene that is expressed in various cell types. It has 2 transcription variants and has dominance over controlling motor neurons.[9]

Delayed rectifier potassium channels are widely distributed in systemic tissues, including skeletal and cardiac muscle. After intracellular sodium ions trigger an action potential, delayed rectifier potassium channels discharge intracellular potassium ions to the outside, functioning to restore the membrane potential to the resting status. *KCNQ1* is the Kv7.1–encoding gene and shows widespread expression in several tissues throughout the body. Kv7.1 regulates key physiological functions and has been implicated in several diseases.[10] *KCNA2* is the Kv1.2-encoding

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gene and is involved in insulin secretion in the pancreas, transportation of electrolytes to the epidermal cells, and muscle contraction.[11]

It has been assumed that hypokalemia in familial hypokalemic periodic paralysis is the result of pooling of potassium ions inside the cell because they cannot be freely discharged; however, the molecular mechanism is yet to be determined.[3] This study was conducted in order to test the hypothesis that the expression levels of major inward and delayed rectifier potassium channel genes in patient cells differ from those in normal cells. We chose to study their expression because of the key role that both types of potassium channels play in regulating the movement of potassium ions through cell membranes.

METHODS

Subjects

We reviewed 178 patients who were being treated for familial hypokalemic periodic paralysis in the Division of Medical Genetics, Department of Pediatrics, Konyang University Hospital. For this study, we selected 3 patients who presented the most severe symptoms. These patients had the Arg1239Gly mutation in *CACNA1S*. Further, 3 healthy individuals participated in this study. All participants provided their written informed consent, and the study was conducted in compliance with the guidelines of the Institutional Review Board of Konyang University Hospital.

Sampling of skeletal muscle specimens

The subjects were asked to rest in a supine position on a bed. Skeletal muscle specimens were collected from the gastrocnemius muscles through surgical incision following local anesthesia with lidocaine.

Preparation of potassium buffers

For exposing cells to normal extracellular potassium concentrations, 4 mM buffer at pH 7.35 (4 mM KCl, 145 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM glucose, and 10 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS)) was prepared. For triggering depolarization of skeletal muscle cells under a high concentration of potassium, 50 mM buffer (50 mM KCl, 145 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM glucose, and 10 mM MOPS) was prepared. Both solutions were sterilized prior to experimental use.

Culturing of skeletal muscle cells and treatment with potassium buffer

The culturing and differentiation of skeletal muscle cells were performed using a protocol previously described.[12] Briefly, after pretreatment, skeletal muscle specimens collected from patients with familial hypokalemic periodic paralysis and healthy volunteers were cultured using Dulbecco's modified Eagle's medium (DMEM; Thermo Scientific, South Logan, UT, USA) containing 20% fetal bovine serum (Thermo Scientific) and 1% penicillin–streptomycin (Thermo Scientific) at 37 °C in an incubator containing 95% air and 5% CO_2 (Thermo Scientific). Thereafter, skeletal muscle cells were cultured in DMEM with 2% horse serum (Thermo Scientific) and 1% penicillin–streptomycin and allowed to differentiate for 5 days. Both the normal and patient cells were collected at the 10th passage and both groups were treated with potassium buffer at normal extracellular concentration (4 mM) and high concentration (50 mM) of potassium in order to induce depolarization, and the mRNA expression levels of inward and delayed rectifier potassium channel genes were analyzed prior to and 1 h following addition of buffer.

Quantitative RT-PCR analysis

Total RNA was isolated from cultured skeletal muscle cells using TriZol (Invitrogen, Carlsbad, CA, USA), and 100 ng was converted to cDNA by using reverse transcriptase. Accupower PCR PreMix (BIONEER, Daejun, Korea) was added to the reaction mix, and quantitative RT-PCR analysis was performed using primers for *KCNJ2*, *KCNJ6*, *KCNJ14*, *KCNQ1* and *KCNA2*. The primer sequences are shown in Table 1. Quantitative measurement of the mRNA was obtained from 10 independent experiments. The expression levels of mRNAs specific for each gene were normalized to the expression of GAPDH.

Statistical analysis

Analyses were performed with the aid of Statistical Package for the Social Sciences (SPSS) 12.0, and results were presented as means ± standard deviation. Comparisons were made using two-way ANOVA. The level of significance was set at $P < 0.05$.

Table 1: PCR Primers for *KCNJ2*, *KCNJ6*, *KCNJ14*, *KCNQ1*, and *KCNA2*

Gene	Primer	Position	Amplicon size (bp)
KCNJ2	F: GTCCCCAATCACTATAGTCC R: GTGTCCGTACTAGTGCTTTC	1175 – 1194 1588 – 1569	413
KCNJ6	F: GGAAATTGTGGTCATCCTAG R: CTTTGGATTCATTCTCCAGG	1443 – 1462 1804 – 1785	361
KCNJ14	F: GCCATGACCACACAGTGTCG R: ATCATGGAGGCAGGGTCAGC	1336 – 1355 1717 – 1698	381
KCNQ1	F: TAAGGAAGAGCCCAACACTG R: CAGGAAGAGCTCAGGGTCGA	1269 – 1288 1837 – 1818	568
KCNA2	F: ATGAGCGAGAGTCCCAGTTC R: GTTAGCCAAGGTACAGTTGG	1551 – 1570 1954 – 1935	403

RESULTS

mRNA expression of inward rectifier potassium channel genes

We used quantitative RT-PCR to examine mRNA levels of potassium channel genes in skeletal muscle cells from both normal and patient groups, prior to and after exposure to 4 mM and 50 mM potassium buffers. When cells from the control and patient groups were exposed to 4 mM potassium buffer (i.e., normal extracellular concentrations), no quantitative change in mRNA expression of treated cells was observed relative to that of untreated cells (data not shown). After exposure to 50 mM potassium buffer, which was used to induce depolarization, mRNA expression in the normal cells was found to be lower (*KCNJ2* 59.4 ± 17.8%, *KCNJ6* 83.7 ± 4.6%, and *KCNJ14* 70.7 ± 10.2%, $P < 0.05$) (Figure 1A, B, and C). However, in patient cells, no significant change in mRNA expression was observed upon exposure to 50 mM buffer (Figure 1A and B) for *KCNJ2* or *KCNJ6*. Interestingly, *KCNJ14* showed increased expression in patient cells (122.3 ± 2.1%, $P < 0.05$) (Figure 1C).

mRNA expression of delayed rectifier potassium channel genes

After exposure to 4 mM potassium buffer, no change in the mRNA level of *KCNQ1* or *KCNA2* was observed in control or patient cells (data not shown). Similarly, neither gene in the normal cells showed any detectable changes in expression following exposure to 50 mM potassium buffer (Figure 2A and B). However, at this high potassium concentration, mRNA expression for cells from the patient group decreased (*KCNQ1* 78.6 ± 4.1% and *KCNA2* 71.5 ± 5.6%, $P < 0.05$) (Figure 2A and B) relative to that of untreated cells.

DISCUSSION

Inward rectifier potassium channels are essential for stabilizing the resting membrane potential, modulating repolarization to a resting potential, and coupling the metabolic cellular state with membrane excitability. In contrast, delayed rectifier potassium channels are primarily involved in repolarization following depolarization of the cell membrane. Delayed rectifier potassium channels are activated by the influx of sodium ions and counteract its effect by allowing the discharge of potassium ions. Familial hypokalemic periodic paralysis has been found to be caused by mutations in *CACNA1S* and *SCN4A*, but the mechanism of the origin of hypokalemia during paralytic attacks has not yet been determined. Recently, through the molecular study of skeletal muscle cells from patients with this disease, we found that hypokalemia may be attributable to potassium channel abnormalities.[12] In the present study, we further examined this possibility by investigating the variation in gene expression for major inward and delayed rectifier potassium channels in normal and patient groups.

Quantitatively, no changes in mRNA expression for *KCNJ2*, *KCNJ6*, and *KCNJ14* were observed for either group at normal concentrations of extracellular potassium (4 mM). These results may indicate that there is no dysfunction in either the intracellular or extracellular movement of potassium ions in the course of depolarization to repolarization in the patient group. This is consistent with patients having no difficulty

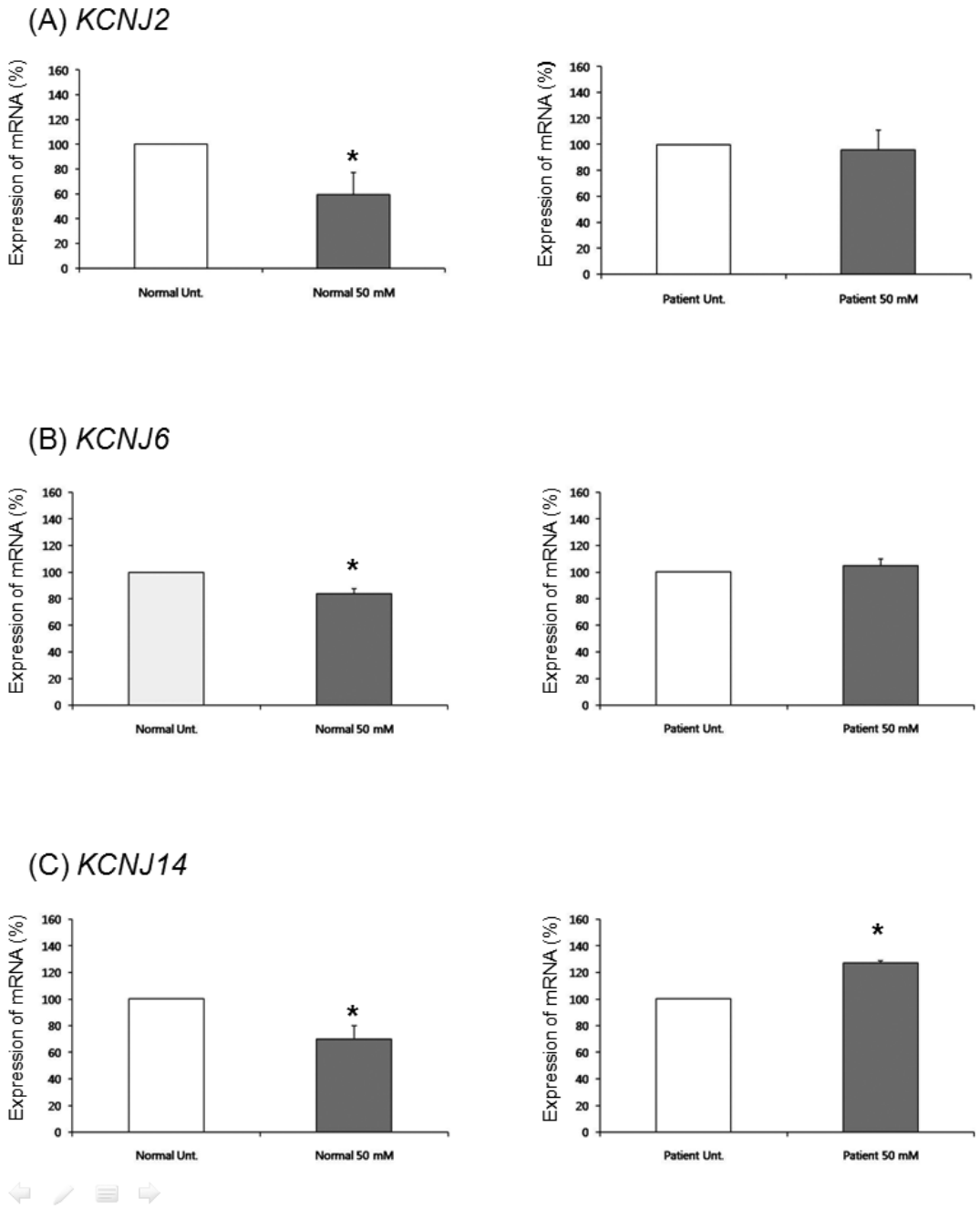

Figure 1. Quantification of *KCNJ2*, *KCNJ6* and *KCNJ14* mRNA expression after exposure to 50 mM potassium buffer. (A) The *KNCJ2* mRNA levels significantly decreased in normal cells, but remained unchanged in patient cells. (B) The *KCNJ6* mRNA levels significantly decreased in normal cells, but remained unchanged in patient cells. (C) The *KCNJ14* mRNA levels significantly decreased in normal cells, but increased in patient cells. Unt (Untreated): prior to exposure to potassium buffer. Treated: 1 h after exposure to 50 mM potassium buffer. Values are expressed as a percentage of the untreated control level. * $P < 0.05$, vs. untreated samples.

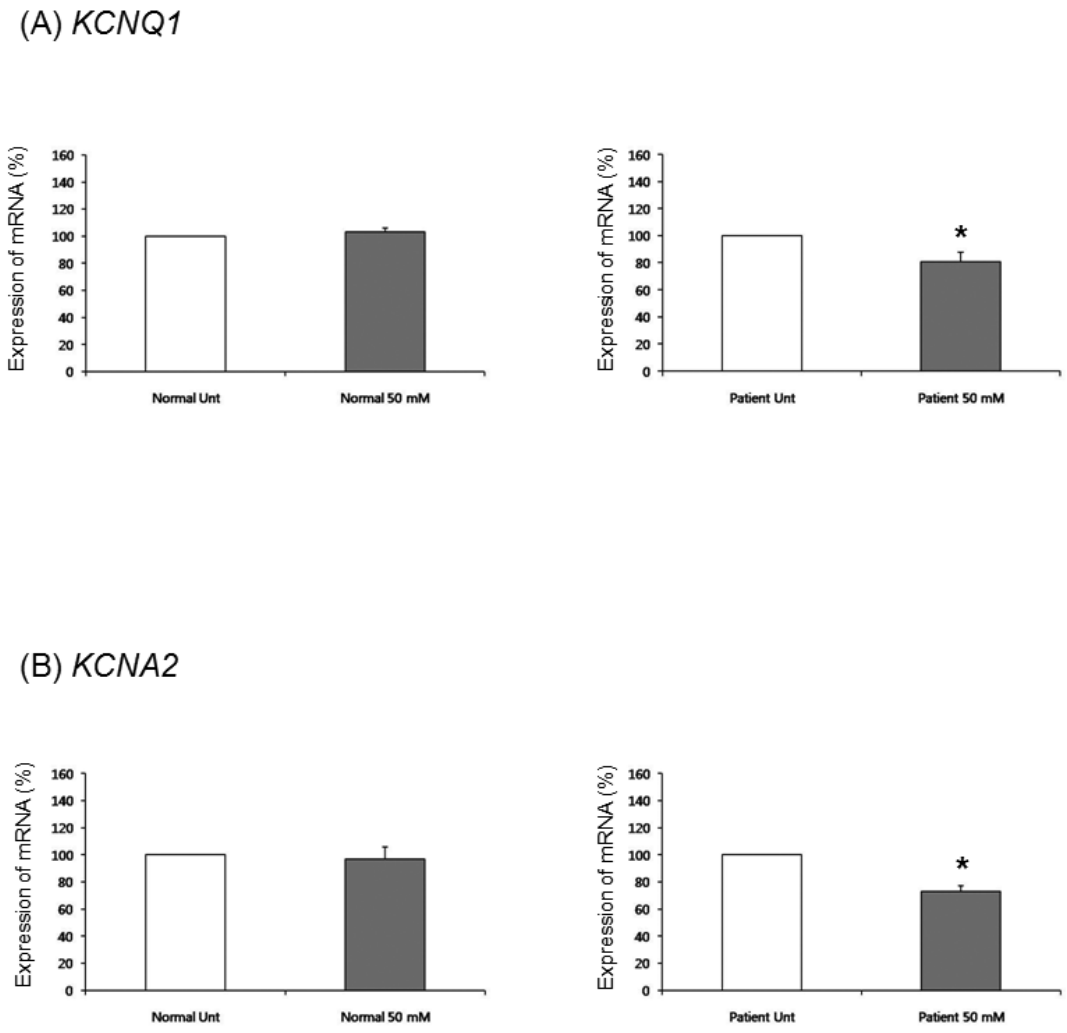

Figure 2. Quantification of *KCNQ1* and *KCNA2* mRNA expression after exposure to 50 mM potassium buffer. (A) The *KCNQ1* mRNA levels remained unchanged in normal cells, but significantly decreased in patient cells. (B) The mRNA levels of *KCNA2* remained unchanged in normal cells but significantly decreased in patient cells. Unt (Untreated): prior to exposure to potassium buffer. Treated: 1 h after exposure to 50mM potassium buffer. Values are expressed as a percentage of the untreated control level. * $P < 0.05$, vs. untreated samples.

with ordinary activities interictally. However, while mRNA expression was reduced for all 3 genes in normal cells at 50 mM potassium concentration, *KCNJ2* and *KCNJ6* showed no expression changes for patient cells, whereas *KCNJ14* showed an increased expression level. These results imply that reduced expression of inward rectifier potassium channel genes in depolarized normal cells may help discharge potassium ions out of the cell, which is then followed by repolarization. On the contrary, in patient cells, unreduced or overexpressed inward rectifier potassium channel genes are inferred to be so dysfunctional that intracellular potassium ions are not discharged but pooled inside, resulting in the obstruction of repolarization and ultimately leading to hypokalemia and paralysis. A similar gene expression pattern of *KCNQ1* and *KCNA2* was observed between normal and the patient cells upon treatment with 4 mM potassium buffer. This suggests that both groups are not different from each other in terms of repolarization following depolarization. However, after exposure to 50 mM buffer, mRNA expression of *KCNQ1* and *KCNA2* showed no quantitative differences in the normal group, but was reduced in the patient group. Potentially, this reduction in gene expression obstructs intracellular potassium ions from discharging outside, resulting in a prolonged state of depolarization and ultimately leading to clinical hypokalemia and paralysis. Future work using electrophysiological analyses in protein levels will further explore this possible mechanism of hypokalemia and may reveal how a calcium channel gene mutation can lead to altered expression of other genes.

In conclusion, this study examined the expression patterns of potassium channel genes in skeletal muscle cells of patients with familial hypokalemic periodic paralysis. We observed that the expression levels of both inward (*KCNJ2*, *KCNJ6*, and *KCNJ14*) and delayed rectifier (*KCNQ1* and *KCNA2*) potassium channel genes in patient cells are different from those in normal cells in response to high extracellular potassium concentrations. These findings could be important in understanding the mechanism that causes the development of hypokalemia in this disorder.

DISCLOSURE

The authors of this article have no conflict of interest.

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REFERENCES

1. Kim JB, Kim MH, Lee SJ, *et al*. The genotype and clinical phenotype of Korean patients with familial hypokalemic periodic paralysis. *J Korean Med Sci* 2007; 22:946-51.
2. Venance SL, Cannon SC, Fialho D, *et al*. The primary periodic paralyses: diagnosis, pathogenesis and treatment. *Brain* 2006; 129:8-17.
3. Struyk AF, Cannon SC. Paradoxical depolarization of BA^{2+}-treated muscle exposed to low extracellular K+: insights into resting potential abnormalities in hypokalemic periodic paralysis. *Muscle Nerve* 2008; 37:326-37.
4. Abraham MR, Jahangir A, Alekseev AE, *et al*. Channelopathies of inwardly rectifying potassium channels. *Faseb J* 1999; 13:1901-10.
5. Kubo Y, Adelman JP, Clapham DE, *et al*. International Union of Pharmacology. LIV. Nomenclature and molecular relationships of inwardly rectifying potassium channels. *Pharmacol Rev* 2005; 57:509-26.
6. Yang D, Zhang X, Hughes BA. Expression of inwardly rectifying potassium channel subunits in native human retinal pigment epithelium. *Exp Eye Res* 2008; 87:176-83.
7. Derst C, Karschin C, Wischmeyer E, *et al*. Genetic and functional linkage of Kir5.1 and Kir2.1 channel subunits. *FEBS Lett* 2001; 491:305-11.
8. Sakura H, Bond C, Warren-Perry M, *et al*. Characterization and variation of a human inwardly-rectifying-K-channel gene (KCNJ6): a putative ATP-sensitive K-channel subunit. *FEBS Lett* 1995; 367:193-7.
9. Tennant BP, Cui Y, Tinker A, *et al*. Functional expression of inward rectifier potassium channels in cultured human pulmonary smooth muscle cells: evidence for a major role of Kir2.4 subunits. *J Membr Biol* 2006; 213:19-29.
10. Jespersen T, Grunnet M, Olesen SP. The KCNQ1 potassium channel: from gene to physiological function. *Physiology* 2005; 20:408-16.
11. Gutman GA, Chandy KG, Grissmer S, *et al*. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* 2005; 57:473-508.
12. Kim SJ, Lee YJ, Kim JB. Reduced expression and abnormal localization of the KATP channel subunit SUR2A in patients with familial hypokalemic periodic paralysis. *Biochem Biophys Res Commun* 2010; 391:974-8.