Molecular cloning of ion channels in *Felis catus* that are related to periodic paralyses in man: a contribution to the understanding of the genetic susceptibility to feline neck ventroflexion and paralysis

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**ABSTRACT**

Neck ventroflexion in cats has different causes; however, the most common is the hypokalemia associated with flaccid paralysis secondary to chronic renal failure. In humans, the most common causes of acute flaccid paralysis are hypokalemia precipitated by thyrotoxicosis and familial forms linked to mutations in sodium, potassium, and calcium channel genes. Here, we describe the sequencing and analysis of skeletal muscle ion channels in *Felis catus* that could be related to periodic paralyses in humans, contributing to the understanding of the genetic susceptibility to feline neck ventroflexion and paralysis. We studied genomic DNA from eleven cats, including five animals that were hyperthyroid with hypokalemia, although only one presented with muscle weakness, and six healthy control domestic cats. We identified the ion channel orthologs *KCNJ2*, *KCNJ12*, *KCNJ14*, *CACNA1S* and *SCN4A* in the *Felis catus* genome, together with several polymorphic variants. Upon comparative alignment with other genomes, we found that *Felis catus* provides evidence for a high genomic conservation of ion channel sequences. Although we hypothesized that neck ventroflexion in cats could be associated with a thyrotoxic or familial periodic paralysis channel mutation, we did not identify any previously detected human channel mutation in the hyperthyroid cat presenting hypokalemia. However, based on the small number of affected cats in this study, we cannot yet rule out this molecular mechanism. Notwithstanding, hyperthyroidism should still be considered as a differential diagnosis in hypokalemic feline paralysis.

**KEY WORDS:** Potassium channel, Inward rectifier, *Felis catus*, Kir2.x, *KCNJ2*, *KCNJ12*, *KCNJ18*, *CACNA1S*, *SCN4A*, Cat

**INTRODUCTION**

Ion channels are macromolecular protein complexes that are components of the cell membrane and are essentially important in different types of signaling, including transport, excitability, and conduction. Alterations in these channels may dynamically disturb cell and tissue physiology (Hille, 2001).

Indeed, there are many diseases related to ion channel dysfunction. In general, dominant and recessive mutations in genes encoding channels may lead to electrophysiological dysfunction, resulting in hyper- or hypo-excitability of the corresponding cells and typically causing so-called channelopathies (Abraham et al., 1999; Rolim et al., 2010; Jongsma and Wilders, 2001; Souto, 2011). Such altered genes can give rise to different clinical manifestations through gain-of-function (enhance) or loss-of-function (attenuate) mutations that affect the channel activity. Certain congenital disturbances affecting the skeletal muscle have been identified in humans and animals, such as disorders in calcium and potassium channels that can lead to paralysis and disorders in chloride channels that can lead myotonia; additionally, both paralysis and myotonia can originate from sodium and calcium channel disequilibrium.

Hypokalaemic Periodic Paralyses (HypokPP) include several uncommon diseases with clinical presentation characterized by acute and reversible attacks of muscle weakness, especially of the lower extremities, associated with low serum potassium. The most prevalent causes of HPP are Familial Hypokalaemic Periodic Paralysis (FHypokPP), an autosomal dominant disease, and an acquired form Thyrotoxic Hypokalaemic Periodic Paralysis (THypokPP), secondary to any cause of thyrotoxicosis. The symptoms of paralysis and the grade of hypokalaemia are almost identical in both FHypokPP and THypokPP, the differences in clinical features are related to the signs of thyrotoxicosis present in THPP (Rolim et al., 2010). Although work in human medicine has broadened the clinical spectrum by identifying several new channel mutations, such molecular genetic research on paralysis is seldom reported in veterinary medicine, and a promising feline animal model of THypokPP would be attractive for further genetic studies.

*Felis catus* is an interesting species for the study of human diseases. There are many instances in which the relationship between clinical signs, etiological agents, and molecular analysis of different pathologies has been established in cats (O’Brien et al., 1997a; O’Brien et al., 1997b), and the possible amino acid and gene sequence conservation among species throughout evolution may allow the identification of orthologous and syntenic genes that share a common evolutionary origin (Navratilova and Becker, 2009; Nomiyama et al., 2013; Ohno, 1973), therefore this animal model would shed light on the understanding of similar skeletal muscle diseases between man and cat.

Indeed, a comparison of genomes and analysis of synteny among ion channels using PCR, cloning, and sequence alignment would be useful for the molecular diagnosis of feline channelopathies. Here, we describe the channel genes *KCNJ2*, *KCNJ12*, *KCNJ14*, *SCN4A*, and *CACNA1S* in *Felis catus* in an attempt to associate the

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Received 19 February 2014; Accepted 17 June 2014
single-nucleotide polymorphisms (SNPs) found in these genes with feline ventroflexion and muscle paralysis.

RESULTS AND DISCUSSION
We were able to study eleven cats including five hyperthyroid animals with hypokalemia, with only one presenting with muscle weakness, and six healthy control domestic cats, as summarized in Table 2. Since, familial hypokalemic periodic paralysis (FHypokPP) is an autosomal dominant disease associated with mutations in calcium channels CACNA1S (Nav1.4) (Kim et al., 2011; Sternberg et al., 1993), and sporadic/thyrotoxic hypokalemic paralysis is related to mutations in KCNJ18 (Kir2.6) (Cheng et al., 2011; Maciel et al., 2011; Ryan et al., 2010; Silva et al., 2004; Wang et al., 2006), we approached principally these genes. The KCNJ18 mutants are primarily associated with THypokPP, an acquired genetic susceptibility condition in human.

Molecular cloning of fKir2.1, fKir2.2 and fKir2.6 (or the human-like KCNJ2, KCNJ12 and KCNJ18 genes)
Of the neuromuscular disorders, cervical ventroflexion is a classic sign of generalized neuromuscular weakness in cats that can have different causes (Dickinson and LeCouteur, 2004). Cats, particularly the Burmese and Siamese breeds, exhibit paralysis associated with hypokalemia and muscle weakness similar to THypokPP in humans (Table 3) and has been termed hypokalemic periodic polymyopathy (HypoPP), sporadic feline hypokalemic polymyopathy, or periodic muscle weakness (Jones and Gruffydd-Jones, 1990; Lantinga et al., 1994). These conditions appear to be related to a sudden influx of potassium from the extracellular to intracellular compartment that is associated with THypokPP crisis in humans to the human-like fKir2.1 (KCNJ2), fKir2.2 (KCNJ12), and fKir2.6 (KCNJ18) from feline genomic DNA using human primers with low-stringency PCR and sequencing. We found that human Kir2.6 shares 96–99% amino acid identity with Kir2.2 and human Kir2.2 shares over 70% identity at the amino acid level with Kir2.1 (Ryan et al., 2010). We also found that the relationship between the two genes is preserved in cats and that the sequences are highly conserved between the species.

PCR amplification using primers designed for hKir2.1 gene yielded a product of approximately 750 bp when examined on a 1% agarose gel (Fig. 1A). Its sequence represents the second exon of fKir2.1 (KCNJ2) gene; this fragment included the feline coding region of the predicted human-like KCNJ2 harboring 222 amino acids, which was then construed from the human sequence (GenBank: NG_008798). Primers designed based on the hKir2.2 gene yielded a product of approximately 625 bp (Fig. 2A), which sequencing revealed that this fragment represents the third exon, comprising part of the coding region of fKir2.2 (KCNJ12) gene, encoding 195 residues, which was also inferred to the human sequence (GenBank: NW_003315950.1).

Through the alignment of feline-like KCNJ2 (750 bp) and KCNJ12 (625 bp) segments in the NCBI Felis catus genome

**Table 1. Details of primers used in this study for PCR, cloning and sequencing assays**

| Gene       | Exon_primer | Sequence                  | MT    |
|------------|-------------|---------------------------|-------|
| KCNJ2      | Ex2_F       | 5'-CTGGAGTTCCACACGACGAAG-3' | 55.5  |
|            | Ex2_R       | 5'-GATACAGAGCATTTGAGTGAAG-3' | 53.9  |
|            | Ex2_F       | 5'-GCTAGTGGCTCTGAGGTGTC-3'  | 55.8  |
|            | Ex2_R       | 5'-TGGGCTTCTTGTACCAACAGA-3' | 56.9  |
| KCNJ12/18  | Ex3_F       | 5'-TGGGCTGTGGTTCTCCATTAAG-3' | 54.4  |
|            | Ex3_R       | 5'-CTCCACAGATGGTGCTTCTAC-3'  | 55.2  |
|            | Ex3_F       | 5'-GAGCTCTGCTGTCTGACGC-3'   | 56.4  |
|            | Ex3_R       | 5'-CGGGCGGAGATGCTT-3'       | 55.6  |
| KCNJ4      | Ex2_F       | 5'-CTGGCGGCTCTCTTCCTCCACG-3' | 60.6  |
|            | Ex2_R       | 5'-CCTCCAGGCCGACTCTTGGGAAG-3' | 61.5  |
|            | Ex2_F       | 5'-CCCCCAAGGCTCTGATCATG-3'  | 60.8  |
|            | Ex2_R       | 5'-CAGAGGCGCTGTCGGGAAG-3'  | 61.1  |
| KCNJ14     | Ex2_F       | 5'-CGCTGATGTGCCGCTTCTC-3'  | 61.5  |
|            | Ex2_R       | 5'-GAGCTTACGTCCTCAGACCCTCCG-3' | 63.1  |
|            | Ex3_F       | 5'-CCGCTGCTCTGGTCGCCTGG-3'  | 61.8  |
|            | Ex3_R       | 5'-GGGAGGGGAAGGGTAAAAATGGC-3' | 61.5  |
| SCN4A      | Ex12_F      | 5'-CTCCCTGAGGCTGCTATGAC-3'  | 61.5  |
|            | Ex12_R      | 5'-GGGTCTCTACTCTCTTGACCC-3' | 61.5  |
|            | Ex18_F      | 5'-TCTAAAGGCTCTGGACCTCC-3'  | 61.6  |
|            | Ex18_R      | 5'-CCCCCGATCCCTAGCCTAC-3'  | 59.5  |
|            | Ex22_F      | 5'-TGAGAGCAGAGAAGGGACC-3'  | 61.5  |
|            | Ex22_R      | 5'-GGTTCTAGAGGAGGGCCAGAGG-3' | 61.4  |
|            | Ex24_F      | 5'-CCTCTCTCCAAGGGGACATG-3'  | 61.6  |
|            | Ex24_R      | 5'-CATAGGGAGGCTTGGGGGCTG-3' | 61.6  |
| CACNA1S    | Ex11_F      | 5'-CGGGTGCCAGAAGGGAAAGT-3'  | 62.2  |
|            | Ex11_R      | 5'-CAGAGGCTGACCAAGGGAGGAC-3' | 61.9  |
|            | Ex30_F      | 5'-CGTGTAGAGGCTCCACCTTACG-3' | 62.3  |
|            | Ex30_R      | 5'-GCGAGCCTCCTCCAGTACG-3'  | 62.2  |

Abbreviations: F, Forward; R, Reverse; MT, melting temperature
The Kir channel family is encoded by the \( \text{KCNJ} \) genes (supplementary material Figs S1, S2, S3) in the \( \text{KCNJ}12 \) genes and among species. With regard to f\( \text{KCNJ}2 \) conservation (Fig. 1C, Fig. 2C) among the cat Kir2.x family orthologues available in the NCBI database, revealing high conservation (Fig. 1C, Fig. 2C) among the cat Kir2.x family orthologues available in the NCBI database, revealing high gene expression. For example, two different subunits of Kir channels are tetrameric (i.e. four subunits), each containing three transmembrane domains (M1, M2, and M3) and a fourth loop (H2) that includes the pore-forming region. The Kir channel family is encoded by the \( \text{KCNJ} \) genes (Dhamoon and Jalife, 2005) and includes seven subfamilies (Kir1-7) sharing 40–60% homology within each (de Boer et al., 2010). Different genetic diseases have been recognized in these channels: Andersen–Tawil syndrome caused by mutations in Kir2.1 (encoded by \( \text{KCNJ}2 \)) (Obeyesekere et al., 2011; Plaster et al., 2001); cardiac cell, neuron, and muscle diseases caused by mutations in Kir2.2 (encoded by \( \text{KCNJ}12 \)) (Hugnot et al., 1997; Prüss et al., 2005; de Boer et al., 2010); and susceptibility to thyrotoxic hypokalemic periodic paralysis (THypokPP) (Ryan et al., 2010), a clinical condition characterized by reversible attacks of muscle weakness associated with thyrotoxicosis, hypokalemia, and hypophosphatemia, caused by mutations in Kir2.6 (T354M, K366R, Q407X, and R399X) (encoded by \( \text{KCNJ}18 \)) (Dias Da Silva et al., 2002; Maciel et al., 2011; Rolim et al., 2010; Ryan et al., 2010).

**Sequencing of hotspot regions for mutations causing human paralysis in the \textit{Felis catus} genome: the \text{SCN}4A and \text{CACNA1S} genes**

Familial hyperkalemic periodic paralysis (HYPPP) is an autosomal dominant channelopathy generated by mutations in sodium channel \( \text{SCN}4A \) (Nav1.4) (Han and Kim, 2011; Ptáček et al., 1991), similar to HYPPP in horses (Alemán, 2008). Other clinical manifestations of skeletal muscle identified to date are potassium-aggravated myotonia (PAM), paramyotonia congenita (PMC), hypokalemic periodic paralysis/familial hypokalemia periodic paralysis (HypoPP/FP), and a form of congenital myasthenic syndrome (CMS). Using PCR with \textit{in silico}-predicted feline primers, we were able to identify and confirm the \( \text{SCN}4A \) cat gene, specifically in relation to regions identified in association with several periodic paralysis mutations, including exons 12 (GenBank: KF267755), 18 (GenBank: KF267756), 22 (GenBank: KF267757), and 24 (GenBank: KF267758) (Fig. 4). Although no mutations were found with regard to divergent amino acid sequences compared to human, two polymorphisms were found in exons 12 and 24 (Table 4).

By targeting hotspot regions in the human \( \text{CACNA1S} \) gene (GenBank: NM_001038605), we investigated the corresponding
exons 11 and 30 in the cat genome, identifying polymorphisms in exon 11, as depicted in Table 4 and Fig. 5. As both CACNA1S (Cav1.1) and SCN4A (Nav1.4) are related to HypoPP/FPP, autosomal dominant disorders causing either muscle weakness or flaccid paralysis with incomplete penetrance and occurring more frequently in young males, with later onset observed in affected females (Lin et al., 2005), we searched for mutations in our group of cats affected with muscle weakness. Although mutations typically affect segment S4 of domains II, III, and IV of CACNA1S and domains I, II, and III of SCN4A (Lin et al., 2010), no mutations in these genes (Table 5) were found in felines.

Channel genes KCNJ2, KCNJ12, SCN4A and CACN1AS appear to be in synteny

Using NCBI tools (http://www.ncbi.nlm.nih.gov/projects/mapview), we were able to draw evolutionary genomic relationships (Fig. 6), as revealed by conservation in sequence identity (>85%), as collinearity by examining the chromosomal locations. The gene group comprising KCNJ12, SCN4A, and KCNJ2 is located on chromosome 17 in humans and chimpanzees, chromosome 11 in mice, and chromosome E1 in cats; the CACNA1S gene is collinearly positioned on chromosome 1 in mice, chimpanzees, and humans and on chromosome F1 in cats. According to O’Brien et al., the syntenic block of feline chromosome E1 corresponds to human chromosome 17 and most likely murine chromosome 11 (O’Brien et al., 1997a); thereby it human 1 chromosome corresponds to C1 feline chromosome, which was reported by Pontius et al. (Pontius et al., 2007).

Our data reinforce the evolutionary structural genomic relationships among channel ortholog genes, which can suggest evolutionary gene duplication and further specialization of channel functioning. With respect to the KCNJ18 gene (Kir2.6), we did not found this gene in either the genome of Felis catus or the Mus musculus C2C12 cell line; thus, we hypothesize that it might also be missing in the other lower mammal species. In fact, we were able to identify in silico Kir2.6 amino acid similarities in the predicted chimpanzee trace genome. Regardless, further studies need to be performed to confirm these findings because of the difficulty in distinguishing between Kir2.6 from Kir2.2 because they share high (98%) genomic homology, thereby limiting the ability to isolate them using ordinary sequencing methods.

Comparative genetic analysis has a high value for evolutionary, biological, and clinical aspects and allows the establishment of animal models for understanding interspecies diseases and

Fig. 1. Identification of the KCNJ2 (Kir2.1) gene in Felis catus. (A) Representative gel electrophoresis of the KCNJ2 PCR product using the cat and human genomes. (B) Chromatogram of the region surrounding glycine 206 used for the comparative analysis (C) against other orthologous genomes available. Note that fKCNJ2 shows 91.1% identity with Homo sapiens (Hs), 90.6% with Canis familiaris (McFarland et al., 1980), 93% with Pan troglodytes (Pt), 91.6% with Bos taurus (Sternberg et al., 1993), 87% with Mus musculus (Prüss et al., 2005), 87.7% with Rattus norvegicus (Rn), and 84.6% with Gallus gallus (Ptaček et al., 1991). Abbreviations: (M) 1-kb molecular marker; cat (C) and human (H) PCR products.

Fig. 2. Identification of the KCNJ12 (Kir2.2) gene in Felis catus. (A) Representative gel electrophoresis of the KCNJ12 PCR product using the cat and human genomes. (B) Chromatogram of the region surrounding glutamine 424 used for comparative analysis (C) against other orthologous genomes available. Note that fKCNJ12 shows 90% identity with Homo sapiens (Hs), 93% with Canis familiaris (McFarland et al., 1980), 90% with Pan troglodytes (Pt), 89% with Bos taurus (Sternberg et al., 1993), 86% with Mus musculus (Prüss et al., 2005), 85% with Rattus norvegicus (Rn), and 78% with Gallus gallus (Ptaček et al., 1991). (D) Five amino acids lacking in the cat sequence (red line); NM_021012 (human sequence) and JX484837 (cat sequence). Abbreviations: (M) 1-kb molecular marker; cat (C) and human (H) PCR products.
### Table 4. Genotype distribution of polymorphic variants found in KCNJ2, KCNJ12, SCN4A and CACN1AS in the group of hyperthyroid cats and control population

| Gene          | Polymorphism | Cat   | Chromosome | Genotype | Allele |
|---------------|--------------|-------|------------|----------|--------|
| **KCNJ2**     | C>A Ser (S) 342 Ser (S) |       |            |          |        |
| C             | 12           | CC    | 8          | 2        | 0.75   | 0.25  |
| HWM           | 8            | CA    | 2          | 2        |        |       |
| HM            | 2            | AA    | 8          | 0.75     | 0.25   |       |
| A>C Ser (S) 373 Ser (S) | |       |            |          |        |
| C             | 12           | AC    | 2          | 4        | 0.4    | 0.6   |
| HWM           | 8            | CC    | 6          | 6        | 0.13   | 0.87  |
| HM            | 2            | AA    | 2          | 2        | 0.5    | 0.5   |
| T>C Ser (S) 413 Ser (S) | |       |            |          |        |
| C             | 12           | TT    | 6          | 6        | 0.25   | 0.75  |
| HWM           | 8            | TC    | 6          | 0.5      | 0.5    |       |
| HM            | 2            | CC    | 2          | 1        |        |       |
| **KCNJ12**    | C>T Arg (R) 149 Arg (R) |       |            |          |        |
| C             | 12           | CC    | 2          | 4        | 0.33   | 0.67  |
| HWM           | 8            | CT    | 4          | 4        | 0.5    | 0.5   |
| HM            | 2            | TT    | 2          | 0.5      | 0.5    |       |
| **CACNA1S_Ex11** | C>A Arg (R) 469 Arg (R) |       |            |          |        |
| C             | 8            | CC    | 2          | 4        | 0.5    | 0.5   |
| HWM           | 8            | CA    | 4          | 0.5      | 0.5    |       |
| HM            | 2            | AA    | 2          | 1        |        |       |
| **SCN4A_Ex12** | C>G Pro(P) 628 Pro (P) |       |            |          |        |
| C             | 12           | CC    | 10         | 2        | 0.92   | 0.08  |
| HWM           | 8            | CG    | 8          | 1        |        |       |
| HM            | 2            | GG    | 2          | 1        |        |       |
| **SCN4A_Ex24** | C>T Leu(L) 1491 Leu(L) |       |            |          |        |
| C             | 12           | CC    | 6          | 6        | 0.75   | 0.25  |
| HWM           | 8            | CT    | 6          | 0.88     | 0.12   |       |
| HM            | 2            | TT    | 2          | 0.5      | 0.5    |       |

Abbreviations: C, control; HWM, hyperthyroidism without muscle weakness; HM, hyperthyroidism with muscle weakness.

**Fig. 3.** Schematic representation of the protein sequence alignment between *Felis catus* Kir2.1 and Kir2.2. (A) Comparative among human and *fKCNJ12* and *fKCNJ2* genes. We can observe 91% and 94% of homology in coding region among species. (B) General scheme of the Kir channel subunits. (C) Putative sequences shared are in bold, showing conserved sequence between subfamily members.
explaining phenotype–genotype relationships. Although we did not discover any polymorphisms with clinical or causal relationships with paralysis/hypokalemia in the cat in this study, the DNA sequences of these genes are available for future studies. In the future, we hope to contribute more genetic studies on the diseases of *Homo sapiens/Felis catus*.

**MATERIALS AND METHODS**

**Feline peripheral blood genomic DNA extraction**

This study was conducted following the guidelines for Ethics Committee of the University Federal of São Paulo registered under CEP 1402/11. We enrolled 11 cats presented to Clinical Veterinary of Small Animal in São Paulo. DNA extraction was performed using a salting-out procedure according to an in-house method, as reported by Kizys et al. (Kizys et al., 2012).

**Felis catus PCR amplification using in silico predicted oligonucleotides**

We initially used human primers previously designed for human potassium channel genes to pull out cat genomic sequence by using low-stringency PCR amplification since their sequence were unknown. The amplifications were performed using 100 ng feline genomic DNA in a 50-μl reaction containing 90% Platinum PCR SuperMix (Invitrogen, Carlsbad, CA). The reaction was as follows: an initial denaturation at 94°C for 2 min, followed by 38 cycles of 94°C for 20 sec, 56°C annealing for 30 sec, and 72°C elongation for 1 min, and a final extension at 72°C for 5 min. For the PCR amplification of the feline SCN4A and CACNA1S genes, primers were designed in silico using NCBI human sequences, as shown in Table 1. These predicted primers were used in a 25-μl PCR reaction containing 100 ng/μl genomic DNA (1 μl) and 22.5 μl Platinum® PCR SuperMix (Invitrogen, Carlsbad, CA, USA); the reaction consisted of 35 cycles at 94°C for 5 min, followed by 94°C for 30 sec, 60°C for 45 sec, and a final extension at 72°C for 10 min. We applied different annealing temperatures for specific exons, as detailed in Table 1.

**Molecular cloning and sequence analysis of feline ion channels**

The expected PCR bands were examined on a 1% agarose gel and then purified. The purified PCR product was cloned into pCR4®TOPO (Invitrogen Carlsbad, CA) and transformed into One Shot® MAX Efficiency® DH5α™-T1® Competent Cells (Invitrogen, Carlsbad, CA). Positive clones were confirmed by sequencing using an ABI Prism 3100 Applied Biosystems Sequencer (CA, USA). The sequences were analyzed using BioEdit Sequence Alignment Editor and CLC Main Workbench 6 (http://www.clcbio.com).

**List of symbols and abbreviations used**

PCR: polymerase chain reaction; SNPs: single-nucleotide polymorphisms; FHypokPP: familial hypokalemic periodic paralysis; ThyroKPP: thyrotropic KPP:

![Fig. 4. Identification of the SCN4A gene in Felis catus. (A) Representative gel electrophoresis of the SCN4A PCR product using the cat genome. We obtained products about for exon 12 (300 bp); exons 18 and 22 (200 bp); exon 24 (1.3 kb). (B–E) Chromatograms representative of exons 12, 18, 22, and 24, respectively. Comparison with other available orthologous genomes. Exon 12: *Homo sapiens* (Hs_97%), *Rattus norvegicus* (Rn_95%), *Mus musculus* (Mm_95%), *Gallus gallus* (Gg_98%), *Canis lupus familiaris* (Cf_97%), *Pan troglodytes* (Pn_97%), and *Bos taurus* (Bt_97%). Exon 18: 100% with *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Canis lupus familiaris*, and *Bos taurus*; *Gallus gallus* (Gg_93%); and *Pan troglodytes* (Pn_96%). Exon 22: *Homo sapiens* (Hs_97%); 100% with *Rattus norvegicus*, *Mus musculus*, *Gallus gallus*, *Canis lupus familiaris*, *Pan troglodytes*, and *Bos taurus*. Exon 24: *Homo sapiens* (Hs_94%); *Rattus norvegicus* (Rn_89%); *Mus musculus* (Mm_88%); *Gallus gallus* (Gg_85%); *Canis lupus familiaris* (Cf_98%); *Pan troglodytes* (Pn_94%); and *Bos taurus* (Bt_93%). Abbreviations: (Ex12) exon 12, (Ex18) exon 18, (Ex22) exon 22, and (Ex24) exon 24.

![Fig. 5. Identification of the CACNA1S gene in Felis catus. (A) Representative gel electrophoresis of the CACNA1S PCR product using the cat genome. (B,C) Chromatograms representative of exons 11 and 30, respectively. Comparison with other available orthologous genomes. Exon 11: *Homo sapiens* (Hs_86%); *Canis lupus familiaris* (Cf_92%); *Pan troglodytes* (Pn_88%); *Bos taurus* (Bt_92%); *Mus musculus* (Mm_88%); *Rattus norvegicus* (Rn_91%); and *Gallus gallus* (Gg_81%). Exon 30: 100% with *Homo sapiens*, *Canis lupus familiaris*, *Pan troglodytes*, and *Rattus norvegicus*; *Mus musculus* (Mm_95%); and *Gallus gallus* (Gg_81%).

![Image](https://via.placeholder.com/150)
Table 5. Comparative skeletal muscle channelopathies observed in mammals

| Channel | Disease                         | Species                                    | Myotonia | Periodic paralysis | Gene   |
|---------|---------------------------------|--------------------------------------------|----------|--------------------|--------|
| Chloride| Myotonia congenita              | Human (Lehmann-Horn and Jurkat-Rott, 2000; Souto, 2011) | X        |                    | CLCN1  |
|         |                                 | Mouse (Vite, 2002)                         | X        |                    |        |
|         |                                 | Goat (Camerino et al., 2000)               | X        |                    |        |
|         |                                 | Dog (Bhalerao et al., 2002; Finnigan et al., 2007; Rhodes et al., 1999) | X        |                    |        |
|         |                                 | Horse (Vite, 2002)                         | X        |                    |        |
|         |                                 | Sheep (Vite, 2002)                         | X        |                    |        |
|         |                                 | Cat (Vite, 2002)                           | X        |                    |        |
| Sodium  | Hyperkalemic periodic paralysis | Human (Lehmann-Horn and Jurkat-Rott, 2000; Souto, 2011; Vite, 2002) | X        | X                  | SCN4A  |
|         |                                 | Dog (Vite, 2002)                           | X        |                    |        |
|         |                                 | Mouse (Vite, 2002)                         | X        |                    | SCN4A  |
|         |                                 | Horse (Aleman, 2008; Finno et al., 2009)  | X        | X                  | SCN4A  |
|         | Paramyotonia congenita          | Human (Lehmann-Horn and Jurkat-Rott, 2000; Souto, 2011; Vite, 2002) | X        | X                  | SCN4A  |
|         | K+-aggravated myotonia (PAM)    | Human (Lehmann-Horn and Jurkat-Rott, 2000; Souto, 2011; Vite, 2002) | X        | X                  | SCN4A  |
| Calcium | Hypokalemic periodic paralysis  | Human (Lehmann-Horn and Jurkat-Rott, 2000; Souto, 2011; Vite, 2002) | X        | X                  | CACNA1S|
|         |                                 | Mouse (Vite, 2002)                         | X        | X                  | Cacna1s|
|         |                                 | Cat (Dickinson and LeCouteur, 2004; Dow et al., 1987; Lantinga et al., 1998; Vite, 2002) | X        |                    |        |
|         |                                 | Dog (Vite, 2002)                           | X        |                    |        |
|         | Malignant hyperthermia          | Human (Vite, 2002)                         | X        | X                  | CACNA1S/CACNL2A |
|         |                                 | Cat (Dickinson and LeCouteur, 2004)       | X        |                    |        |
|         |                                 | Dog (Vite, 2002)                           | X        |                    |        |
| Potassium| Andersen–Tawil syndrome         | Human (Vite, 2002)                         | X        |                    | KCNJ2  |
|         | Hypokalemic periodic paralysis  | Human (Vite, 2002)                         | X        |                    |        |
|         |                                 | Cat (Dickinson and LeCouteur, 2004; Dow et al., 1987; Lantinga et al., 1998; Vite, 2002) | X        |                    |        |
|         |                                 | Dog (Vite, 2002)                           | X        |                    |        |
|         | Hypokalemic thyrotoxic periodic paralysis| Human (Ryan et al., 2010; Tricarico and Camerino, 2011) | X        |                    | KCNJ18 |
|         |                                 | Cat (Dickinson and LeCouteur, 2004)       | X        |                    |        |
|         | Hyperkalemic periodic paralysis | Human (Vite, 2002)                         | X        |                    | KCNE3  |
|         | Malignant hyperthermia          | Human (Vite, 2002)                         | X        |                    | RYR1   |
|         |                                 | Horse (Aleman, 2008; Finno et al., 2009)  | X        |                    |        |
|         | Central core disease            | Human (Lehmann-Horn and Jurkat-Rott, 2000; Souto, 2011) | X        |                    | RYR1   |

*Molecular basis unknown. Data modified and organized by the authors based on references (Vite, 2002; Souto, 2011).*
Abdou-Filha, lab assistants Fernando A. Soares, Susan C. Lindsey, Ana Luiza R. Rolim, Krishna R. O. Sousa, Teresa S. Kasamatsu, João R. M. Martins, Maria Clara C. Melo, Maria Izabel Chiamolera, Marina Kizys, Carolina W. Xavier, Kelen C. Oliveira, and Gilberto K. Furuzawa for daily technical assistance, and Angela Faria for secretarial support.

Competing interests

The authors have no competing interests to declare.

Author contributions

M.Z. has conducted the entire design and execution of experiments, as well as the interpretation of the findings, article’s drafting and revision of the article’s proof. I.S.K. and R.M.P. participated in performing lab experiments and data analysis. D.M.N.S. and A.R. Jr contributed with the initial project concept, diagnosis of hyperthyroidism and refereeing the affected cats for ion channel molecular study. V.A.C. participated in the conception and interpretation of the findings and in drafting the paper. R.M.B.M. contributed with lab experimental resources, drafting and revising the article. As senior researcher and corresponding author, M.R.D.S. participated in the conception, design and interpretation of the findings, article’s drafting, manuscript organization and proof revision.

Funding

The authors are supported by a research grant and scholarship from the São Paulo State Research Foundation (FAPESP) 2011/20747-8 for M.R.D.S. and 2012/02529 for M.Z., respectively.

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