# Lack of association of the potassium channel–associated peptide MiRP2-R83H variant with periodic paralysis

D. Sternberg, MD, PhD; N. Tabti, MD, PhD; E. Fournier, MD; B. Hainque, PhD; and B. Fontaine, MD, PhD

**Abstract—**A missense variant (R83H) of the gene (KCNE3) encoding a potassium channel–associated peptide, MinK-related peptide 2 (MiRP2), has been reported in periodic paralysis patients. In the current study, no difference in the frequency of the MiRP2-R83H variant between periodic paralysis patients and healthy individuals was found. Furthermore, there was no segregation of this gene variant with the disease. These observations weaken the proposal that MiRP2-R83H causes periodic paralysis.

NEUROLOGY 2003;61:857–859

Periodic paralyses are disorders of the skeletal muscle characterized by episodic attacks of muscle weakness caused by abnormal membrane excitability.[1,2] They are classified as hyperkalemic (hyperPP) or hypokalemic (hypoPP) periodic paralysis according to variations in blood potassium levels during the attacks. Both sporadic and genetic forms of these diseases have been described. The better-characterized sporadic form is hypoPP with thyrotoxicosis, which is more prevalent in people of Asian descent. The recognized genetic forms of periodic paralysis are autosomal dominant. Most dominant hyperPP cases are caused by missense mutations in the *SCN4A* skeletal muscle voltage-gated sodium channel gene, whereas most hypoPP cases are due to missense mutations in *CACNA1S* skeletal muscle voltage-gated L-type calcium channel gene (hypoPP1; approximately 70% of the cases) or in *SCN4A* gene (hypoPP2; approximately 10% of the cases).[3] The *SCN4A* mutations associated with hyperPP or hypoPP are distinct and induce different biophysical defects of the voltage-gated sodium channel. Andersen's syndrome is another form of dominant periodic paralysis associated with cardiac and dysmorphic signs and is due to missense or in-frame deletions of the *KCNJ2* inwardly rectifying potassium channel gene.[4]

A missense variant in the *KCNE3* gene encoding MinK-related peptide 2 (MiRP2), a potassium channel–associated peptide, was reported in 2 of 100 independent cases with periodic paralysis.[5] A transition from G to A was identified at position 340 of *KCNE3* mRNA, predicting an arginine-to-histidine amino acid substitution at position 83 of MiRP2 peptide (MiRP2-R83H variant). The variant segregated with the disease in two small pedigrees and was absent from 120 control subjects. In a distinct study, 1 patient of 15 with thyrotoxic hypoPP carried the MiRP2-R83H variant.[6] These reports suggested that the MiRP2-R83H variant caused hereditary periodic paralyses and predisposed to thyrotoxic hypoPP. Following up these observations, we studied the frequency of the MiRP2-R83H variant in patients with periodic paralysis and in a large group of 506 healthy control subjects. We also analyzed the segregation of this variant with the disease.

**Patients and methods.** We investigated a total of 104 patients (Group 1) for whom the diagnosis of periodic paralysis was established. Based on clinical and genetic analyses, we could divide this group of patients into two subgroups: A and B. Subgroup A consisted of 64 probands with either hypoPP or hyperPP who were unsuccessfully screened for mutations in *SCN4A* and *CACNA1S* genes. It is worth noting that seven of these patients had hypoPP with thyrotoxicosis. Subgroup B comprised 40 hypoPP patients with identified mutations in *SCN4A* (R669H, n = 1; R672H, n = 7; R672G, n = 1; R672S, n = 1) or *CACNA1S* (R528H, n = 23; R1239H, n = 7). The control group (Group 2) consisted of 506 healthy individuals.

DNA was extracted from blood samples obtained after informed consent of each individual according to European Union and French bioethics laws. PCR amplification of the DNA fragment encompassing site 340 of the *KCNE3* gene was performed as described.[3] DNA fragments were analyzed using single-strand conformation polymorphism analysis (SSCP) denaturing high-pressure liquid chromatography (DHPLC). The SSCP screening procedure included a denaturation step and migration at 7 °C. The DHPLC was performed on a DHPLC WAVE 2100A system (Transgenomic, Omaha, NE). Abnormal DNA products were further characterized by direct sequencing following a new PCR amplification.

From the Federations of Biochemistry (Drs. Sternberg and B. Hainque), Physiology (Drs. Tabti and Fournier), and Neurology (Dr. Fontaine), Centre hospitalier universitaire Pitié-Salpêtrière (Assistance publique-hôpitaux de Paris et Université Paris VI), Faculty of Pharmaceutical Sciences (Dr. Hainque), Université Paris V, and INSERM U546 Research Unit (Drs. Tabti and Fontaine), Paris, France.

Supported by INSERM, AFM, AP-HP, and RESOCANAUX.

Received March 24, 2003. Accepted in final form May 12, 2003.

Address correspondence and reprint requests to Dr. B. Fontaine, INSERM U546, 105 boulevard de l'hôpital, 75013 Paris, France; e-mail: bertrand.fontaine@psl.ap-hop-paris.fr

 

***Table*** *Frequency of MiRP2-R83H variant in periodic paralysis patients and in healthy controls*

| Parameter | Periodic paralysis patients: Group 1 | | Healthy controls: Group 2 |
|---|---|---|---|
| | Subgroup A | Subgroup B | |
| MiRP2-R83H variants | 0 | 1 | 8 |
| Total no. | 64 | 40 | 506 |
| Frequency | 0.0096 | | 0.0158 |

Frequency of MiRP2-R83H variant did not differ between periodic paralysis and control groups according to Yates' corrected $\chi^2$ test ($p = 0.98$).

**Results.** We estimated the frequency of the MiRP2-R83H variant in a large group of periodic paralysis patients, using SSCP or DHPLC screening techniques. Our results showed that only 1 patient of 104 carried this variant (table), which yields a frequency of 0.0096. This patient belonged to Subgroup B (hypoPP with *SCN4A* mutations) and carried both MiRP2-R83H variant and *SCN4A*-R672H mutation, which causes hypoPP2 (figure). Given this result, we screened available members of this family for both MiRP2-R83H and *SCN4A*-R672H. We found that the affected father carried the *SCN4A*-R672H mutation, whereas the healthy mother carried the MiRP2-R83H variant (see the figure). Both father and son presented an unambiguous hypoPP with a decreased blood potassium levels to 2 m*M* on average during attacks of muscle weakness. It is worth noting that the severity of the disease (duration and frequency of the attacks) was comparable between father (carrying *SCN4A*-R672H) and son (carrying both *SCN4A*-R672H and MirP2-R83H). In Subgroup A (hypoPP or hyperPP patients with no *SCN4A* or *CACNA1S* mutation), none of the patients presented the MiRP2-R83H variant. In the group of healthy controls (Group 2), 8 of 506 healthy individuals carried the MirP2-R83H, yielding a frequency of 0.0158. Altogether, our data indicate that the frequency of MiRP2-R83H variant obtained in patients with periodic paralysis (0.0096) is not different from that observed in healthy control subjects (0.0158) (see the table).

**Discussion.** This study demonstrates that the frequency of the MiRP2-R83H variant obtained from patients with periodic paralysis is comparable with that obtained from a large group of healthy control subjects. It is noteworthy that the only patient carrying MiRP2-R83H variant also carried a well-characterized hypoPP2 *SCN4A* mutation. In contrast to MiRP2-R83H variant, this hypoPP2 *SCN4A* variant clearly segregated with the disease. Altogether, our results argue against a causative role of the MiRP2-R83H variant in periodic paralyses.

In the original report, the MiRP2-R83H variant was associated with either hyperPP- or hypoPP-like phenotypes,[5] which weakens the hypothesized causative link between genotype and phenotype. Indeed, up to now, approximately 30 missense mutations in the *SCN4A* and 3 in the *CACNA1S* genes have been described, and none of them has been linked to both phenotypes.[1,2] One may therefore not exclude that, alike our *SCN4A*-R672H patient, patients reported by Abbott and et al[5] carry, in addition to MiRP2-

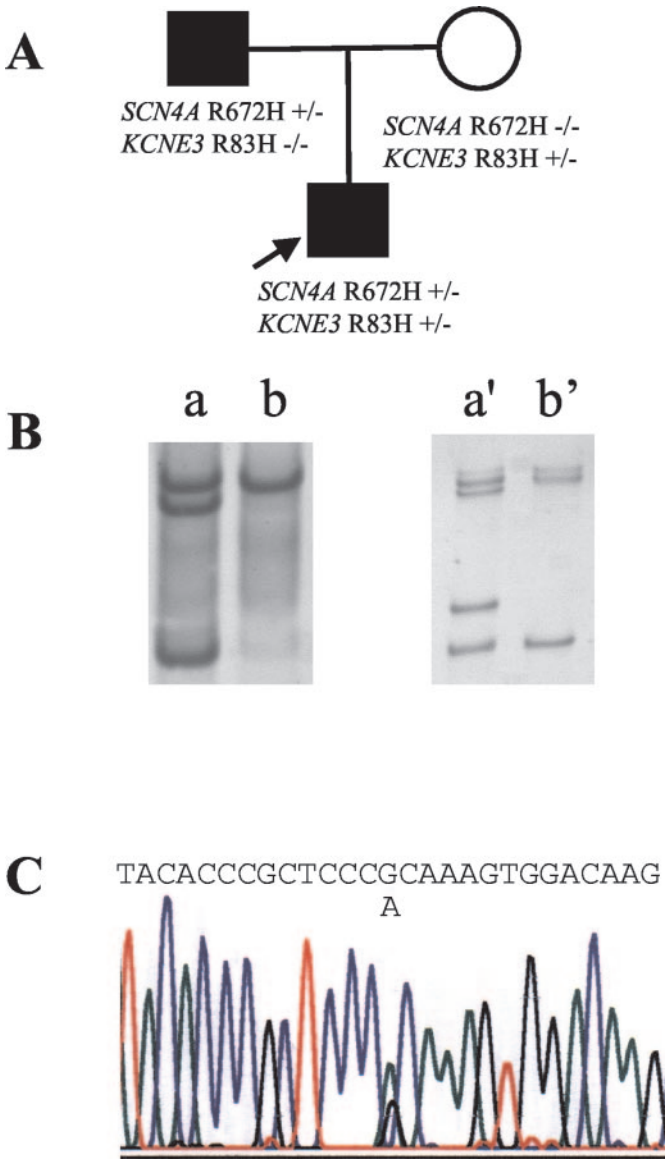


*Figure. A family with hypokalemic periodic paralysis (hypoPP2). (A) Pedigree of a hypoPP family (hypoPP2). Father and son display hypoPP. Both have a sodium channel* SCN4A *mutation (R672H+/−), causing hypoPP2. Healthy mother carries a potassium channel–associated peptide* KCNE3-*R83H variant (R83H+/−). The son is a double heterozygote for* SCN4A-*R672H and* KCNE3-*R83H (arrow).* SCN4A-*R672H is inherited from the father and* KCNE3-*R83H from the mother. (B) Single-strand conformational polymorphism analysis profiles of PCR-amplified* SCN4A *exon 12 region (a: patient [R672H]; b: control) and* KCNE3 *3' coding region (a': patient [R83H]; b': control). (C) Electrophoretogram obtained after direct sequencing of the* KCNE3 *3' coding region amplified by PCR from patient's blood DNA. A G-to-A transition at nucleotide 340 is predicted to cause an arginine-to-histidine amino acid change at position 83 of the protein MiRP2 encoded by the* KCNE3 *gene.*

R83H variant, other distinct hyperPP or hypoPP mutations that remained unidentified despite the efforts of the authors.

In the same study, the authors investigated the functional consequences of MiRP2-R83H in heterologous expression systems.[5] They hypothesized that MiRP2 operates with Kv3.4 potassium channels in skeletal muscle to set the resting membrane potential and that MiRP2-R83H variant may alter this function when co-expressed with wild-type MiRP2. However, a further and more direct confirmation of MiRP2 role in skeletal muscle is needed, as conflicting expression data have been reported for *KCNE3*.[5,7] MiRP2 may indeed interact with different potassium channel subunits, as it has been suggested for MiRP1 in heart.[8-10] The in vivo pathophysiologic relevance of the functional abnormalities induced by MiRP2-R83H when co-expressed in vitro with Kv3.4 needs, therefore, further evaluation before this variant can be classified as a risk factor for muscle excitability diseases.

## References


1. Cannon SC. An expanding view for the molecular basis of familial periodic paralysis. Neuromusc Dis 2002;12:533–543.
2. Lehmann–Horn F, Jurkat–Rott K, Rudel R. Periodic paralysis: understanding channelopathies. Curr Neurol Neurosci Rep 2002;2:61–69.
3. Sternberg D, Maisonobe T, Jurkat–Rott K, et al. Hypokalaemic periodic paralysis type 2 caused by mutations at codon 672 in the muscle sodium channel gene *SCN4A.* Brain 2001;124:1091–1099.
4. Plaster NM, Tawil R, Tristani–Firouzi M, et al. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. Cell 2001;105:511–519.
5. Abbott GW, Butler MH, Bendahhou S, Dalakas MC, Ptacek LJ, Goldstein SAN. MiRP2 forms potassium channels in skeletal muscle with Kv3.4 and is associated with periodic paralysis. Cell 2001;104:217–231.
6. Dias da Silva MR, Cerutti JM, Arnaldi LAT, Maciel RMB. A mutation in the *KCNE3* potassium channel gene is associated with susceptibility to thyrotoxic hypokalemic periodic paralysis. J Clin Endocrinol Metab 2002;87:4881–4884.
7. Schroeder BC, Waldegger S, Fehr S, et al. A constitutively open potassium channel formed by KCNQ1 and KCNE3. Nature 2000;403:196–199.
8. Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 1999;97:175–187.
9. Tinel N, Diochot S, Borsotto M, Ladzunski M, Barhanin J. KCNE2 confers background current characteristics to the cardiac KCNQ1 potassium channel. EMBO J 2000;19:6326–6330.
10. Zhang M, Jiang M, Tseng GN. MinK-related peptide 1 associates with Kv4.2 and modulates its gating function. Circ Res 2001;88:1012–1019.


CME

# Heart valvular disease in patients with Parkinson's disease treated with high-dose pergolide


G. Van Camp, MD, PhD; A. Flamez, MD; B. Cosyns, MD; J. Goldstein, MD, PhD; C. Perdaens, MD; and D. Schoors, MD, PhD



**Abstract—**The authors report the clinical, echocardiographic, and pathologic findings in two patients treated with more than 5 mg of pergolide daily who developed symptomatic severe heart failure due to restrictive valvular disease. They also describe the echocardiographic data of another eight patients taking similar doses of pergolide presenting no clinical signs of heart failure. The findings suggest a possible role of high doses of pergolide in inducing restrictive valvular disease.



NEUROLOGY 2003;61:859–861


Dopamine agonists are now commonly used in the symptomatic treatment of Parkinson disease (PD). The use of high doses of pergolide, an ergot-derived dopamine agonist, has been proposed in the treatment of advanced PD.[1-3]

Several neurologic disorders have been treated with ergot-derived drugs and their side effects are well documented. The occurrence of pulmonary fibrosis and valvular heart disease in patients with migraine can be related to the use of ergot alkaloid drugs and the echocardiographic and pathologic features have been described.[4,5]

Ergot-induced side effects such as pulmonary fibrosis are rare but have been reported during pergolide treatment.[4] We describe the clinical, echocardiographic, and pathologic findings in 10 patients taking high doses of pergolide, two of them presenting with severe heart failure.

**Methods and patients.** *Case 1.* A 61-year-old man had had PD since 1993. For several years he had been treated with levodopa monotherapy until 5 mg of bromocriptine daily was added 2 years before current referral. Bromocriptine was switched to 4 mg of pergolide after 6 months and the dose was gradually increased to a maximum of 8 mg daily.


From the Departments of Cardiology (Drs. Van Camp, Cosyns, and Schoors), Neurology (Dr. Flamez), Cardiac Surgery (Dr. Goldstein), and Pathology (Dr. Perdaens), Vrije Universiteit Brussels, Belgium.



Received February 23, 2003. Accepted in final form June 3, 2003.



Address correspondence and reprint requests to Dr. G. Van Camp, Department of Cardiology, Free University Brussels (AZ-VUB), Laarbeeklaan 101, 1090 Brussels, Belgium; e-mail: guy.vancamp@az.vub.ac.be