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Abstract—*Background:* Periodic paralysis is classified into hypokalemic (hypoPP) and hyperkalemic (hyperPP) periodic paralysis according to variations of blood potassium levels during attacks. *Objective:* To describe new mutations in the muscle sodium channel gene *SCN4A* that cause periodic paralysis. *Methods:* A thorough clinical, electrophysiologic, and molecular study was performed of four unrelated families who presented with periodic paralysis. *Results:* The nine affected members had episodes of muscle weakness reminiscent of both hyperPP and hypoPP. A provocative test with potassium chloride was positive in two patients. However, repeated and carefully performed tests of blood potassium levels during attacks resulted in normal potassium levels. Remarkably, two patients experienced hypokalemic episodes of paralysis related to peculiar provocative factors (corticosteroids and thyrotoxicosis). Similarly to hyperPP, electromyography in nine patients revealed increased compound muscle action potentials after short exercise and a delayed decline during rest after long exercise as well as myotonic discharges in one patient. With use of molecular genetic analysis of the gene *SCN4A*, three new mutations were found affecting codon 675. They resulted in an amino acid substitution of a highly conserved arginine (R) to either a glycine (G), a glutamine (Q), or a tryptophan (W). Interestingly, hypoPP is caused by both mutations affecting nearby codons as well as the change of an arginine into another amino acid. *Conclusion:* A potassium-sensitive and normokalemic type of periodic paralysis caused by new *SCN4A* mutations at codon 675 is reported.

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Periodic paralysis is a muscle disorder characterized by episodic attacks of muscle weakness. It is classified into hypokalemic (hypoPP) and hyperkalemic (hyperPP) periodic paralysis according to results of blood potassium level measurements obtained during attacks. Features such as the duration, hour of occurrence in the day, and triggering factors of paralytic episodes tend to differ between both types. In hypoPP, attacks last several hours. Typically, the patient awakes paralyzed the morning following strenuous exercise and meals rich in carbohydrates. In hyperPP, episodes of muscle weakness are usually shorter and encountered during the day. They are provoked by cold, rest after exercise, and fasting.

It is now well established that mutations in ion channel genes cause the genetic forms of periodic paralysis. These forms are rare inherited disorders (approximate prevalence: 1/100,000) with an autosomal dominant mode of inheritance. Missense mutations in the voltage-gated calcium channel Cav1.1 result in hypoPP, whereas missense mutations in the voltage-gated sodium channel Nav1.4 give rise to both hyperPP and hypoPP.[1-3] Mutations of the sodium channel that cause hyperPP and hypoPP are distinct.[1-3]

The mutations causing periodic paralysis affect the α-subunit of Nav1.4 or Cav1.1 encoded by the genes *SCN4A* and *CACNA1S*. The α-subunit is made up of four repeated domains (DI to DIV), each of which consists of six transmembrane segments (S1 to S6). The fourth transmembrane segment (S4) of each domain contains positively charged amino acids that sense voltage.[4]

Among the ion channel mutations causing periodic paralysis, a number of them are recurrent and can be used as molecular tools for diagnosis. This is the case for the T704M and the M1592[1-3] as well as the R672H mutations[5,6] of the sodium channel that cause hyperPP and hypoPP. This is also true for the R528H and the R1239H mutations of the calcium channel that give rise to hypoPP.[1-3] Studies of large populations of patients with periodic paralysis have shown that in addition to these classic mutations, which are linked to the well-characterized phenotypes described above, other mutations also exist and may cause unusual types of periodic paralysis.

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These mutations are rare and may be unique to an individual family. Even if rare, these unusual and infrequent types of periodic paralysis are important to study for genetic counseling and to illuminate pathophysiology.

In this context, we describe four families with a potassium-sensitive normokalemic periodic paralysis caused by different mutations at codon 675 of the gene *SCN4A*. We reasoned that it was of interest for neurologists to report this phenotype as mutations that affect nearby codons as well as the change from an arginine into another amino acid both cause hypoPP.

Materials and methods. *Patients.* Patients were examined by one of the authors. All human tissue samples used in this project were obtained after informed consent of each individual according to the European Union and French bioethics laws as well as the Declaration of Helsinki. Periodic paralysis is classified into hypoPP or hyperPP according to variations of blood potassium levels measured during attacks. Therefore, patients were instructed to call a nurse at home when they were paralyzed (paraplegic or quadriplegic) to draw blood and send it immediately to a laboratory. The normal range of the laboratory was 3.0 to 5.5 mmol/L. Some of the patients were challenged by potassium chloride during hospitalization.[1-3] After an EKG was performed, 3 grams followed half an hour later by 8 grams of KCl were given orally. Every half hour, patients were clinically examined and blood potassium levels were measured. The tests were declared positive if the patients displayed an episode of muscle weakness as assessed by a physician and comparable with attacks they reported.

Electrophysiology. Electromyographic (EMG) evaluations were performed using a protocol described in detail in a recent study.[7] In brief, compound muscle action potentials (CMAPs) were recorded by EMG from right and left abductor digiti minimi (ADM) muscles with surface steel disk electrodes taped in place using a belly-tendon configuration. A bandage around the hand prevented articulation displacements and changes in muscle volume during the exercise tests. The appropriate ulnar nerve was supramaximally stimulated at the wrist, using a bipolar bar electrode held in place manually. Between two and five supramaximal CMAPs were recorded at rest to ensure a stable baseline response. Two kinds of exercises were performed: 1) short exercise of the left ADM muscle lasting 10 seconds, with recording of the CMAPs immediately after exercise cessation and then every 10 seconds for 1 minute; 2) long exercise of the right ADM muscle lasting 5 minutes, with brief (3 to 4 seconds) resting periods every 30 to 45 seconds to prevent ischemia. CMAPs were recorded immediately after the 5-minute exercise, every minute for 5 minutes, and then every 5 minutes for 40 minutes. CMAP amplitude, duration, and area were expressed as a percentage of the reference values measured before exercise. Values plotted on the figures and given in the text are means ± SEM. They were compared with values measured using 30 control subjects with the same protocol. The *t*-test was used to compare the group of patients with the group of control subjects. Myotonic discharges were also searched by needle EMG in the deltoid, extensor digitorum communis, first interosseus, vastus medialis, and tibialis anterior muscles.

Molecular diagnosis. We searched for previously described hypoPP and hyperPP mutations in *CACNA1S* (exons 11 and 30) and *SCN4A* (exons 12, 13, and 24) in DNA of probands by previously described techniques.[5] In addition, a new mutation detection system was set up to detect point mutations in exon 13 with more sensitivity. A denaturing high-pressure liquid chromatography (D-HPLC)–based screening procedure was performed on a D-HPLC WAVE 2100A system (Transgenomic, Omaha, NE) as follows: Three microliters of each PCR product was injected on a DNASep cartridge at exactly 63.1 °C, under a loading flow containing 0.1 *M* triethylammonium acetate and 12% acetonitrile (vol/vol). DNA was then eluted from the cartridge at the same temperature by a 0.9-mL/min flow containing a linear gradient of acetonitrile over 4 minutes. DNA elution was monitored by an ultraviolet detection module. All products resulting in D-HPLC profiles different from the homozygous wild-type profile were characterized by direct sequencing after a new PCR amplification.

Results. *Clinical features.* Four families were investigated. Clinical features are summarized in table 1, and pedigrees are shown in figure 1. Family A is a four-generation kindred family with eight affected members (see figure 1A). Five of them were examined (see table 1). Patient AIII8 had experienced attacks of muscle weakness since age 2. The episodes worsened between ages 10 and 20. At this time, he frequently woke up with a generalized paralysis with difficult swallowing and breathing. He was, however, never hospitalized. The paralysis was associated with muscle pains. During an attack, the blood potassium level was measured to fall within the normal range (3.5 mmol/L). The patient was challenged with oral potassium salts as described above. Two hours after potassium ingestion, his potassium blood level increased from 3.8 to 5.2 mmol/L, and he developed tensing of jaws and muscle fatigue similarly to an attack. Clinical examination between attacks showed no myotonia. At age 30, he developed a progressive fixed muscle weakness affecting the proximal upper and lower limbs. Limb proximal weakness was assessed between attacks and ranged in score from 3 to 4 according to the Medical Research Council Rating Scale. Muscle weakness affected all limb muscles but was predominant in the proximal muscle of the lower limbs (hip flexion and knee extension: 3; shoulder abduction and elbow flexion: 4). The patient was treated for 10 years with acetazolamide (250 mg daily) without any beneficial effect, according to him.

Patient AIII1 had presented episodes of muscle weakness since age 3. During childhood and adolescence, the attacks caused quadriplegia. It is worth noting that one of them occurred after general anesthesia. During adulthood, the episodes involved only the lower limbs and were accompanied by stiffness and muscle pains. The patient observed that continuing walking or exercising shortened the attacks. On clinical examination, muscle strength was well maintained without evidence of myotonia. The patient was episodically treated by acetazolamide (1 tablet of 250 mg), with a positive effect on the duration of attack.

Patient AIII3 had noted attacks of weakness since age 15 months. Nocturnal episodes of generalized paralysis with respiratory difficulties were experienced during adolescence. According to the patient, since age 20, she had encountered milder attacks involving only the lower limbs. Continued exercise alleviated muscle weakness. She also noted that stiffness and muscle pains appeared when weakness began to disappear. Clinical examination showed that muscle strength was normal between attacks and that there was no grasp or percussion myotonia. The patient was treated episodically by 250 mg of acetazolamide, which, according to the patient, shortened the attacks.

The attacks of Patient AIV1 started at age 14 months. During childhood, the patient frequently awoke after napping with a generalized paralysis. Since then, she classified her attacks into two types: nocturnal severe episodes of weakness, which appeared only after an intensive exercise (dancing), and exercise- or fasting-induced milder episodes. A blood potassium level measured during a severe attack (quadriplegia) was in the normal range (4.9 mmol/L) although slightly increased over its basal level

Table 1 *Phenotypes of affected individuals in Families A, B, C, and D*

ID no.	Sex (age, y)	Age at onset of paralytic attacks	Frequency of mild paralytic attacks	Duration of mild episodes	Frequency of severe paralytic attacks	Duration of severe episodes	Precipitating factors	Myotonia	Permanent muscle weakness	Muscle pain/ stiffness during attacks	Serum potassium during attack	Muscle biopsy	Medication
AIII1	F (48)	3 y	1/wk	1–48 h	ND	ND	RAE, Imm, stress	−	−	+/+	ND	ND	ACZ
AIII3	F (45)	15 mo	1/mo	Half-day	2–3/y	7 d	RAE, CT, Imm	−	−	+/+	ND	ND	ACZ
AIII8	M (45)	2 y	—	—	2–3/y	15–30 d	RAE, CT, Imm, alcohol	−	+	+/+	NL	VAC, MP	ACZ
AIV1	F (25)	14 mo	1/mo	24 h	4/y	5 d	RAE, Imm, fasting, stress	−	−	+/+	NL	ND	ACZ
AIV2	M (19)	15 mo	4/y	48 h	2/y	1 mo	—	−	−	+/+	NL	ND	ACZ
BIII2	M (40)	18 y	5/wk	20 min	2/y	24–48 h	RAE, fasting	−	−	+/−	ND	ND	—
BIII3	M (38)	16 y	3/wk	2 h	3 in life	<24 h	RAE, cortisone	−	+	+/+	D	NSP	ACZ, potassium, thiazidic diuretics
CIII2	M (26)	23 y	Daily	1–3 h	3–4/wk when thyrotoxic	24 h	RAE, Imm, fasting, CT, cortisone	Lid lag	−	−/+	D, NL	ND	ACZ, potassium
D	M (30)	15 y	4/y	2 wk	6 in life	3 d	RAE, Imm	−	−	−/−	NL	ND	ACZ, potassium

RAE = rest after exercise; Imm = immobility; CT = cold temperature; ND = not determined; NL = normal; D = decreased; MP = myopathic changes; VAC = vacuoles; NSP = nonspecific changes; ACZ = acetazolamide.

(3.9 mmol/L). The patient observed a painful stiffness when the attacks ended. The clinical examination between attacks was unremarkable. The patient was treated discontinuously by 250 mg of acetazolamide, which shortened the attacks.

Patient AIV2 reported what he called severe and mild attacks of weakness since he began to walk. Severe attacks always occurred at night: He would wake up with a generalized weakness and with respiratory and swallowing difficulties. It is notable that the most severe attack occurred at age 10 after general anesthesia and lasted 2 months, during which the patient used a wheelchair. Mild episodes mostly involved the lower limbs and occurred without obvious provocative factors. A potassium blood level was measured during a quadriplegic episode and was found in the normal range (4.1 mmol/L). After recovering from paralysis, the patient noted that weakness was replaced by stiffness and painful muscle cramps. Clinical examination between attacks revealed normal muscle testing despite atrophic shoulders. There was no myotonia. The patient was taking a tablet of 250 mg of acetazolamide to abort attacks.

Two of the three affected members of Family B were examined (see figure 1B and table 1). Patient BIII3 noticed his first attack of paralysis at age 16. Between 16 and 36 years old, he experienced only episodes of muscle fatigue after exercise. These episodes were so mild that they were not brought to medical attention. The patient was able to practice sports at a top level and joined the French professional army. At age 36, he had cervical neuralgia. A few hours after the oral intake of 48 mg of methylprednisolone, he awoke the next morning with a sensation of stiffness in his legs, which progressively turned into a painful weakness. When he was admitted by the end of afternoon to a hospital, he was paraplegic. His blood potassium level was decreased to 2.7 mmol/L. Symptoms resolved in <24 hours following oral potassium supplementation. A similar episode with no obvious precipitating factor occurred 5 months later. The blood potassium level was not measured. Since then, he has had a permanent sensation of fluctuating weakness, stiffness, and pain in the legs associated with mild episodes of weakness occurring at rest after exercise. Several blood potassium levels measurements performed during attacks were in the normal range. Clinical examination between attacks at age 38 showed a global lower limb muscle weakness rated between 3 and 4 using the Medical Research Council Scale (hip flexion and knee extension: 3; shoulder abduction and elbow flexion: 4). There was no myotonia. The patient was sequentially treated with oral potassium salts, thiazide diuretics, and acetazolamide (500 mg/day). None of these treatments modified symptoms. His son (Patient BIV1) was reported to have had one episode of painful cramps and stiffness in the lower limbs at age 14.

Patient BIII2 had his first attack of weakness at 18 years old. During the following 15 years, he experienced severe exercise- or fasting-induced episodes. Since age 3, he has encountered milder daily attacks involving only the lower limbs. Between attacks, the clinical examination was normal.

A single case of proper diagnosis could be documented in Family C (see figure 1C and table 1). The maternal

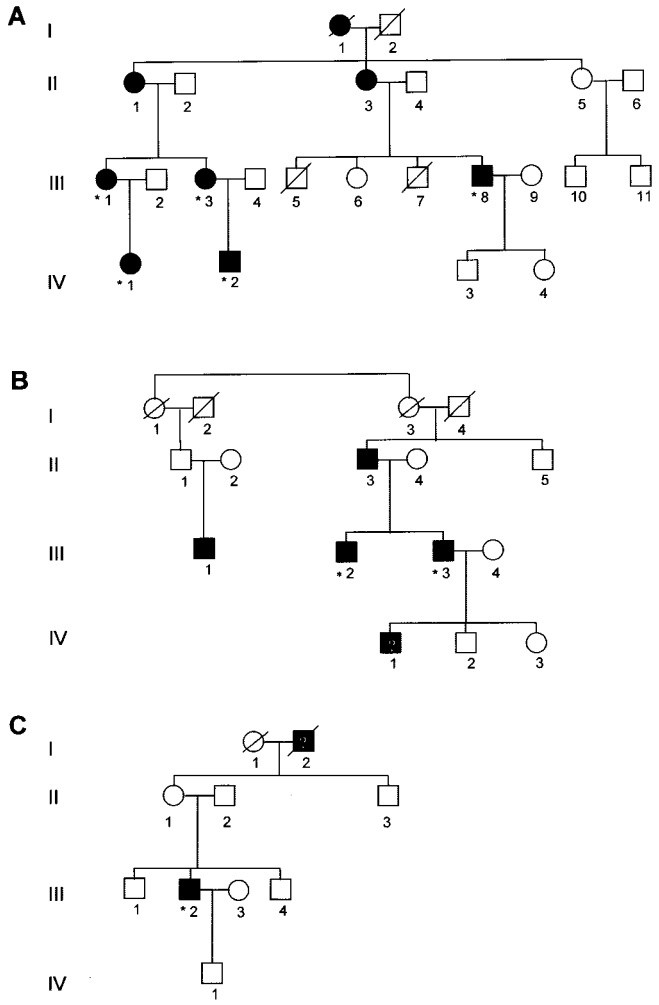

*Figure 1. Pedigrees of the families displaying a potassium-sensitive normokalemic periodic paralysis. (A) Family A; (B) Family B; (C) Family C. Squares = males; circles = females; filled symbols = affected cases; open symbols = unaffected cases; * = case examined.*

grandfather of the index case was reported to have had episodic muscle weakness related to multiple sclerosis. The onset of attacks of Patient CIII2 was at age 23. He had infrequent exercise-induced muscle weakness involving alternatively one or the other lower limb. These mild episodes were considered normal muscle fatigue. At age 24, the patient awoke with paraplegia, which was resolved within a few hours. During the following weeks, he encountered attacks of muscle weakness leading to paraplegia. One month later, laboratory tests revealed a thyrotoxicosis related to Graves disease: Thyroid-stimulating hormone was undetectable, T3 level was 35 ng/L (normal <5 ng/L), T4 level was 100 ng/L (normal <18 ng/L), and antithyroid antibody level was 35 UI/L (normal <1 UI/L). The diagnosis of thyrotoxic hypokalemic periodic paralysis was proposed, although there was no sampling of kalemia. A specific treatment with carbimazole (60 mg/day) was started, and the patient presented with an attack of hives a month later. The patient was ordered to take 48 mg/day of methylprednisolone. The following morning, when he took his first tablets of cortisone, the patient was hospitalized in the emergency room for a life-threatening quadriplegia, which resolved within a few hours. The blood potassium level was measured when the patient was quadriplegic and was found severely decreased at 1.4 mmol/L. Despite normalization of the thyroid function within a few weeks and oral potassium salt supplementation, the patient still had daily episodes of muscle weakness, which started by a sensation of bad smell, paresthesias in the legs, and muscle stiffness. Blood potassium values during one of the paralytic episodes were measured in the normal range (4.1 mmol/L). Between attacks, clinical examination was normal except for a lid-lag sign. The patient was challenged with a potassium chloride solution as described in Materials and Methods. This resulted in a peak potassium level at 6.2 mmol/L 90 minutes after ingestion, with a concomitant loss of strength noted in all limbs, predominating in the lower limbs. The patient was treated by acetazolamide (250 mg/day), which decreased the number and severity of attacks.

Family D is composed of a single case (see table 1). At age 15, Patient D experienced episodes of acute muscle weakness affecting the trunk and the lower limbs, which was resolved within few days. During the following 10 years, he presented five similar episodes occurring with delay after tennis matches or a long airplane flight. Low blood potassium levels just below the normal range were measured during one episode, but results of repeated testing during other episodes were normal. Between attacks, the neurologic examination was normal. During the last 5 years, the patient reported episodes of weakness, without apparent triggering factors that implicated a single part of the body, such as finger flexors muscle weakness after playing guitar. Since the patient has been taken acetazolamide (500 mg/day), he has remained free of attack.

Electrophysiology. EMG evaluations were performed in the nine patients, twice in four of them. In all cases, examinations were performed at least 1 month after the most recent attack of severe paralysis. EMG findings representative of all studied patients are summarized in figure 2 (A and B). After a short exercise test (see figure 2A), CMAP amplitude showed a mean increase of +13.5 ± 1.3%, significantly higher than in control subjects (+4.2 ± 1.2%; $p < 0.001$). After a long exercise test (see figure 2B), no change of CMAPs was observed immediately after exercise. However, a decrease in amplitude and area appeared 15 to 20 minutes after exercise cessation, reaching its lowest point at 40 minutes (amplitude: −29.0 ± 5.7%; area: −40.3 ± 6.1%; $p < 0.001$). Muscle percussion or needle displacement elicited myotonic discharges in all muscles of Patient CIII2 (see figure 2C), whereas no myotonic discharges were observed in the other patients.

Molecular diagnosis. The index cases (AIII8, BIII3, CIII2, and D) of four families were screened for known mutations causing either hyperPP or hypoPP. Screening techniques did not detect any of the previously described mutations in either the *CACNA1S* (exons 11 and 30) or the *SCN4A* (exons 12, 13, and 24) gene in DNA from index cases. Interestingly, the new D-HPLC-based screening method that we developed to study exon 13 of the *SCN4A* gene revealed abnormal profiles in the four index cases (figure 3). Direct sequencing confirmed that all four patients carried heterozygous DNA nucleotide substitutions at codon 675 of the *SCN4A* gene (see figure 3), which resulted in a R675G, a R675Q, and a R675W amino acid

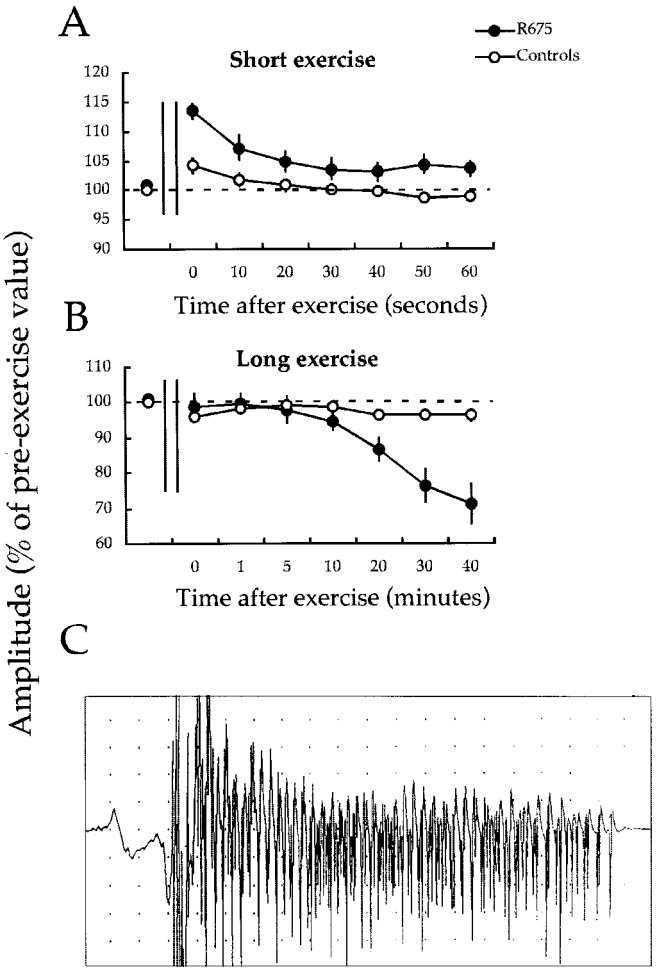

Figure 2. Electromyographic (EMG) study of nine patients with a mutation at codon 675 of the SCN4A *gene. (A, B) The compound muscle action potentials (CMAPs) of the abductor digiti minimi (ADM) were recorded with skin electrodes following the ulnar nerve stimulation at wrist before and after exercise: short exercise of the left ADM (A) and long exercise of the right ADM (B). Amplitudes of CMAPs expressed as a percentage of its pre-exercise value are plotted against the time elapsed after the exercise trial (noted by pairs of vertical bars). (C) Myotonic discharge recorded by needle EMG in Patient CIII2 after muscle percussion. Filled circles = means of the measurements performed on the nine patients; open circles = mean changes of CMAP amplitudes in 30 control subjects; vertical bars = 1 SEM.*

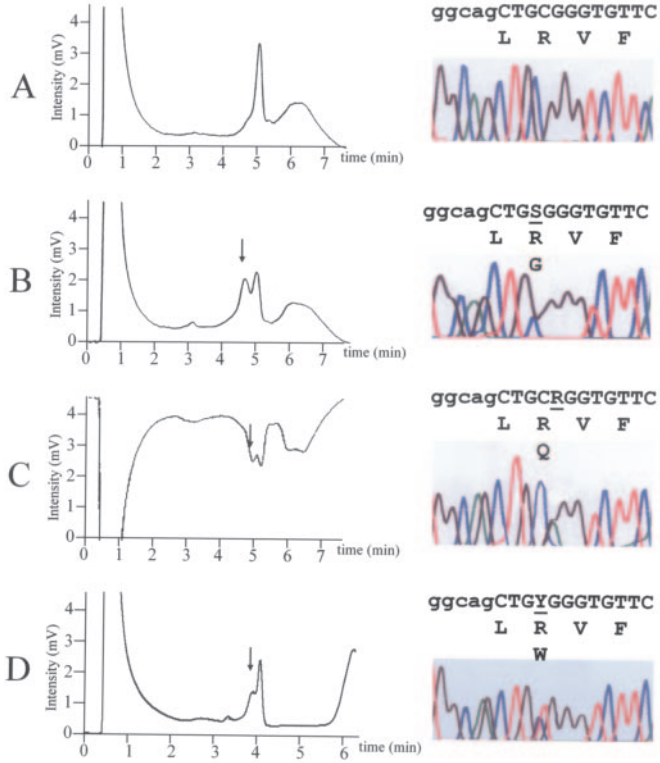

Figure 3. Denaturing high-performance liquid chromatography (D-HPLC) detection and sequence determination of variants at codon 675 variants of SCN4A *gene. Each lane shows the D-HPLC elution profile and the details of forward sequence. Lane A = normal control; lane B = Patient AIII8; lane C = Patient BIII3; lane D = Patient CIII2. For the elution graph, the x-axis represents time (minutes) and the y-axis signal intensity (mV). Note that the time scale of the D-HPLC profile in graph D is different from that in graphs A, B, and C because it was obtained on a different D-HPLC apparatus. The vertical arrow above elution profiles indicates the additional peak containing heteroduplexes, which suggests the presence of a heterozygous mutation. In the sequence, the heterozygous sites are noted according to International Union of Biochemistry codes (S = G, C; R = A, G; Y = C, T).*

change in Patients AIII8, BIII3, and CIII2. Patient D displayed a R675Q substitution similar to Patient BIII3.

The same screening procedure was applied to the four affected relatives of Patient AIII8 (AIII1, AIII3, AIV1, and AIV2), to the asymptomatic parents of Patient D, and to the affected brother of Patient BIII3 (BIII2). The amino acid changes were observed in all five affected relatives as well as in the asymptomatic mother of Patient D.

The same procedure was applied to a population of 254 healthy control subjects. None of them presented an abnormal profile. Thus, none of the amino acid changes observed in patients was present in control subjects.

Discussion. We report new *SCN4A* mutations that cause a potassium-sensitive normokalemic type of periodic paralysis. Nine patients, belonging to four different families and carrying three distinct missense mutations at codon 675 of the muscle sodium channel gene *SCN4A*, were thoroughly studied.

Five patients noticed that attacks were induced by fasting or cold temperatures. Two patients were aware that continued exercise shortened the attacks. Myotonia was never present on clinical examination between attacks, although one patient had a lid-lag sign. It is, however, likely that myotonia played a part at the beginning or at the end of the attacks as the patients had stiffness and painful cramps in the affected limbs. These clinical features suggested the diagnosis of hyperPP.

The diagnosis of hyperPP was further supported by EMG exploration. In the nine tested patients, ex-

ercise tests showed an increase in CMAP amplitude after short exercise and a delayed decline during rest after long exercise. Combined with the presence of myotonic discharges, these EMG abnormalities resembled those we have recently described as pattern IV in a large EMG study of patients with myotonia or periodic paralysis caused by known ion channel defects. Pattern IV was characterized by rare myotonic discharges, a gradual increase of CMAPs after short exercise, and an early increase followed by a late decrease of CMAP amplitude in the long exercise test. Pattern IV was observed in 83% of the patients with hyperPP caused by the T704M sodium channel mutation.[7] Two features distinguished the EMG pattern of patients with a sodium channel mutation at codon 675 from pattern IV and elementary EMG abnormalities previously described in hyperPP patients.[7-9] The decline in CMAP amplitude from baseline values (−29%) was milder in patients with a sodium channel mutation at codon 675, and this decline was not preceded by any amplitude increase immediately after long exercise. To account for the increased CMAP amplitude after short exercise and for the delayed decline after long exercise, one can hypothesize that with an increase in exercise duration, muscle membrane switches from an increased to a decreased excitability, leading to paralysis. Accordingly, exercise was described as a provocative factor of attacks.

It is noteworthy that repeated and careful measurements of blood potassium levels during attacks were normal in the five analyzed patients. There is a debate about the existence of normokalemic periodic paralysis. In families described as having normokalemic periodic paralysis, molecular diagnosis has, indeed, shown that they carried either T704M or M1592V mutation of *SCN4A,* which are previously described as causing hyperPP.[10] Accordingly, in a large series of patients with the T704M *SCN4A* mutation, increased blood potassium levels measured during attacks in similar conditions as in this study were observed in only 50% of cases.[11] In patients carrying a mutation at codon 675 of the gene *SCN4A*, blood potassium levels above the normal range were not observed during attacks despite careful search. It is, however, remarkable that for Patient AIV1, an increased blood potassium level was observed during an attack, although it never crossed the threshold of the upper value of the normal range. In addition, all patients challenged with potassium chloride responded positively to the test. Our observations therefore do not argue in favor of the hypothesis that normokalemic periodic paralysis and hyperPP are distinct disorders but that the expression of hyperPP is variable.

It is striking that two patients (BIII3, CIII2) experienced severe attacks of weakness with decreased blood potassium values. One of them (CIII2) had thyrotoxicosis. A severe attack occurred after the ingestion of a single dose of cortisone for both of them. Thyrotoxic periodic paralysis is a well-established entity, and this diagnosis was proposed for Patient CIII2. Attacks of periodic paralysis usually cease when the thyroid function is normalized. Unexpectedly, attacks persisted for Patient CIII2 despite a normal thyroid function. This led us to re-evaluate the diagnosis. The common precipitating factor of hypokalemic attacks in the two patients was the ingestion of a single dose of cortisone. If a chronic treatment with corticoids may cause decreased blood potassium levels due to renal loss of potassium, this mechanism cannot be used to explain hypokalemia after the ingestion of a single dose of cortisone. It is worth noting that hypokalemia was more severe in the patient who also had thyrotoxicosis. It is well known that both cortisone and thyroid hormones have a profound effect on muscle membrane excitability and may thus participate in the initiation of an attack.[12,13] We report herein their effects on potassium fluxes in patients with a sodium channel mutation. This effect might not be specific to the mutation affecting codon 675 of *SCN4A* as exposure of periodic paralysis patients to either cortisone or thyroid hormones is a rare occurrence. One tempting hypothesis would be that cortisone and thyroid hormones, on the one hand, and regular attacks, on the other, drive potassium fluxes in opposite directions. It is well known that both corticoids and thyroid hormones activate the Na^+-K^+ pump, which is a central target for regulation of membrane excitability.[13]

In the nine studied patients, D-HPLC analysis showed abnormal conformers in exon 13 of the voltage-gated sodium channel gene *SCN4A*. These sequence alterations were heterozygous, segregated with the disease, and were not found in a large panel

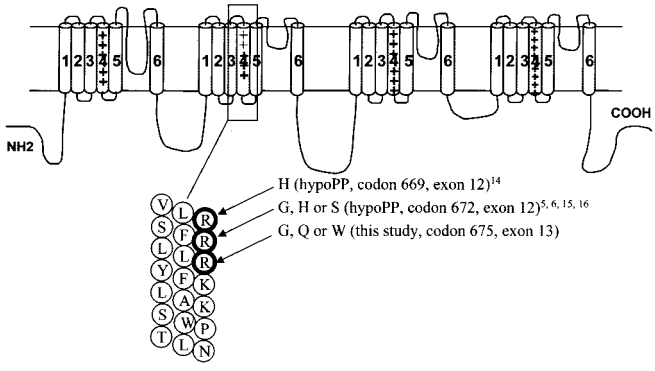

Figure 4. Schematic model of the α-subunit of the muscle sodium channel, showing positions of missense mutations found in the fourth segment of domain II. The α-subunit is made up of four repeated domains (I to IV), each of which consists of six transmembrane segments (S1 to S6). The amino acid sequence of the S4 segment of domain II is shown below. The three amino acid changes found at codon 675 in this study affect the third arginine (R) of the segment, which is replaced by either a glycine (G), a glutamine (Q), or a tryptophan (W) (noted by arrow). The previously reported DII/S4 mutations, affecting the two other arginines located above, are also shown.[5,6,14-16] HypoPP = hypokalemic periodic paralysis.

Table 2 *Periodic paralysis and mutations in muscle voltage-gated sodium channel gene* SCN4A

Amino acid change	Domain/ segment	Phenotypes	Ref.
R669H	D2/S4	HypoPP	14
R672H/G	D2/S4	HypoPP	5, 6
R672S	D2/S4	HypoPP	5, 15, 16
L689I	D2/S4–S5	HyperPP	17
T704M	D2/S5	HyperPP	18
		HyperPP/PC	19, 20
A1156T	D3/S4–S5	HyperPP/PC	21, 22
P1158S	D3/S4–S5	Cold-induced hypoPP + myotonia	23, 24
M1360V	D4/S1	HyperPP	25, 26
		HyperPP/PC	27
M1370V	D4/S1	HyperPP/PC	28
R1448C	D4/S4	HyperPP/PC	29
R1448H	D4/S4	HyperPP/PC	30
F1490L + M1493I	D4/S5	HyperPP	31
I1495F	D4/S5	HyperPP	32
M1592V	D4/S6	HyperPP	33, 34
		HyperPP/PC	35

HypoPP = hypokalemic periodic paralysis; HyperPP = hyperkalemic periodic paralysis; PC = paramyotonia congenita.

of controls, which suggests that they were not benign polymorphisms but instead disease-causing mutations. They resulted in an amino acid substitution of a highly conserved arginine (R) to either a glycine (G), glutamine (Q), or tryptophan (W). The R675G, R675Q, and R675W mutations were localized in the membrane spanning segment S4 of domain II, which is known to be involved in the voltage sensing of the channel (figure 4). To date, 15 mutations causing periodic paralysis have been identified in the muscle sodium channel gene *SCN4A* (table 2).[5,6,14-35] It is worth noting that all mutations causing hypoPP in the muscle sodium channel gene *SCN4A* (R669H, R672G/H/S) affect the two arginines of segment S4 of domain II located above the one encoded by codon 675 (see figure 4).[5,6,14-16] Similarly, an arginine-to-histidine (R528H) mutation in the voltage-gated calcium channel gene *CACN1AS* at the homologous position of the R669H mutation of the muscle sodium channel *SCN4A* gene is a common cause of hypoPP. In contrast, the two mutations affecting the outermost arginine of S4 segment of domain IV (R1448C/H) lead to paramyotonia congenita and in some families to mixed features of hyperPP and paramyotonia congenita (see table 2).[29,30,36] Expression studies and in vitro electrophysiologic recordings of muscle fibers of patients with hypoPP mutations in *SCN4A* or *CACN1AS* have suggested a loss of function mechanism that is proposed to account for the attacks of paralysis. These studies did not, however, shed light on the mechanisms of potassium fluxes observed in hypoPP. Expression studies of sodium channel mutants at codon 675 in the presence of thyroid hormones or cortisone may help to elucidate mechanisms regulating potassium fluxes in periodic paralysis.

Finally, it is important to note that, that in contrast to a majority of hypoPP patients harboring a mutation at codon 672 of *SCN4A*,[5,16] six of the patients with a mutation at codon 675 were improved by treatment by acetazolamide. There was no effect for two of them. None of them worsened.

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