

# A recessive Na<sub>v</sub>1.4 mutation underlies congenital myasthenic syndrome with periodic paralysis



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## ABSTRACT

**Objective:** To determine the molecular basis of a complex phenotype of congenital muscle weakness observed in an isolated but consanguineous patient.

**Methods:** The proband was evaluated clinically and neurophysiologically over a period of 15 years. Genetic testing of candidate genes was performed. Functional characterization of the candidate mutation was done in mammalian cell background using whole cell patch clamp technique.

**Results:** The proband had fatigable muscle weakness characteristic of congenital myasthenic syndrome with acute and reversible attacks of most severe muscle weakness as observed in periodic paralysis. We identified a novel homozygous *SCN4A* mutation (p.R1454W) linked to this recessively inherited phenotype. The p.R1454W substitution induced an important enhancement of fast and slow inactivation, a slower recovery for these inactivated states, and a frequency-dependent regulation of Na<sub>v</sub>1.4 channels in the heterologous expression system.

**Conclusion:** We identified a novel loss-of-function mutation of Na<sub>v</sub>1.4 that leads to a recessive phenotype combining clinical symptoms and signs of congenital myasthenic syndrome and periodic paralysis, probably by decreasing channel availability for muscle action potential genesis at the neuromuscular junction and propagation along the sarcolemma. *Neurology*® 2016;86:161-169

## GLOSSARY

**AP** = action potential; **CMS** = congenital myasthenic syndromes; **ENMG** = electroneuromyography; **NMJ** = neuromuscular junction; **PP** = periodic paralysis; **SCN4A** = sodium channel, voltage-gated, type 4,  $\alpha$  subunit gene; **WT** = wild-type.

Defective propagation of muscle action potential (AP) has been demonstrated in hyperkalemic/normokalemic or hypokalemic periodic paralyses (PP), a group of autosomal dominant diseases characterized by attacks of muscle weakness concomitant to fluctuation of blood potassium levels.<sup>1,2</sup> PP are due to gain-of-function mutations in the skeletal muscle voltage-gated sodium (Na<sub>v</sub>1.4) and calcium (Ca<sub>v</sub>1.1) channels that eventually result in a functional unavailability of Na<sub>v</sub>1.4.<sup>3</sup> Defective generation of muscle AP at the neuromuscular junction (NMJ) leads to congenital myasthenic syndromes (CMS). This heterogeneous group of rare diseases results from functional or structural synaptic changes due to mutations in 22 genes with expression restricted or not to the NMJ (see references 4 and 5 for a review). Missense substitutions of the sodium channel, voltage-gated, type 4,  $\alpha$  subunit gene (*SCN4A*), which encodes the pore-forming  $\alpha$  subunit of Na<sub>v</sub>1.4, were reported 12 years ago in a single patient with a severe form of CMS.<sup>6</sup> The patient was heteroallelic for the p.V1442Q substitution that severely impaired Na<sub>v</sub>1.4 fast inactivation and the p.S246L one. The latter was classified as a polymorphism by the authors based on their functional investigations, suggesting that the disease was dominantly inherited. The second patient with CMS linked to *SCN4A* was reported only recently.<sup>7</sup>

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Interestingly, the disease was recessively inherited and was due to a loss-of-function missense mutation (p.R1457H) that enhanced fast inactivation of the channel.

We report a patient with a mixed phenotype of CMS and PP that is due to a recessive missense mutation of *SCN4A*, and investigate the gating behavior of the mutant channel to fit it with this unusual phenotype.

**METHODS Standard protocol approvals, registrations, and patient consents.** The study had ethical approval from a national ethics committee (DC-2012-1535 and AC-2012-1536). All patients gave written informed consent for genetic testing.

**Patient investigations.** The patient and her parents were investigated neurophysiologically on a Nihon Kohden (Nakano-ku, Japan) machine (Neuropack S1 standard) using standardized protocols.<sup>8,9</sup> Genetic testing was done by bidirectional Sanger sequencing of PCR-amplified coding regions and intron–exon boundaries on genomic DNA extracted from blood. Pathogenicity prediction was done with AlamutVisual (Interactive Biosoftware, Rouen, France). NM\_000334.4 was used as reference for *SCN4A*.

**Plasmid constructs and mutagenesis.** The p.R1454W mutation was introduced into the wild-type (WT) human  $\alpha$  subunit cDNA sequence of  $\text{Na}_v1.4$  in the pRcCMV-*SCN4A* plasmid using the Quikchange II site directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). The final cDNA construct was sequenced to ensure the presence of the expected mutation and the integrity of the rest of the plasmid sequence.

**Cell culture, transfection, and electrophysiology.** Human embryonic kidney cells (tsA201) were routinely grown in Dulbecco modified Eagle medium (PAA, Les Mureaux, France) supplemented with 10% fetal calf serum (PAA), 1% penicillin-streptomycin, and 0.5 mM L-glutamine, and maintained at 37°C with 5%  $\text{CO}_2$ . The calcium-phosphate precipitation technique was employed for transient cell transfection.<sup>10</sup> The WT or mutated cDNA was cotransfected with pEGFP. Recordings were conducted at room temperature (24°C) in the whole-cell configuration as previously described.<sup>11</sup> Additional protocols are described in the supplementary material on the *Neurology*<sup>®</sup> Web site at [Neurology.org](http://Neurology.org).

**Statistical analyses.** Values represent means  $\pm$  SEM and n the number of cells tested. Statistical data were obtained using Student *t* test with  $p < 0.05$  considered as significant.

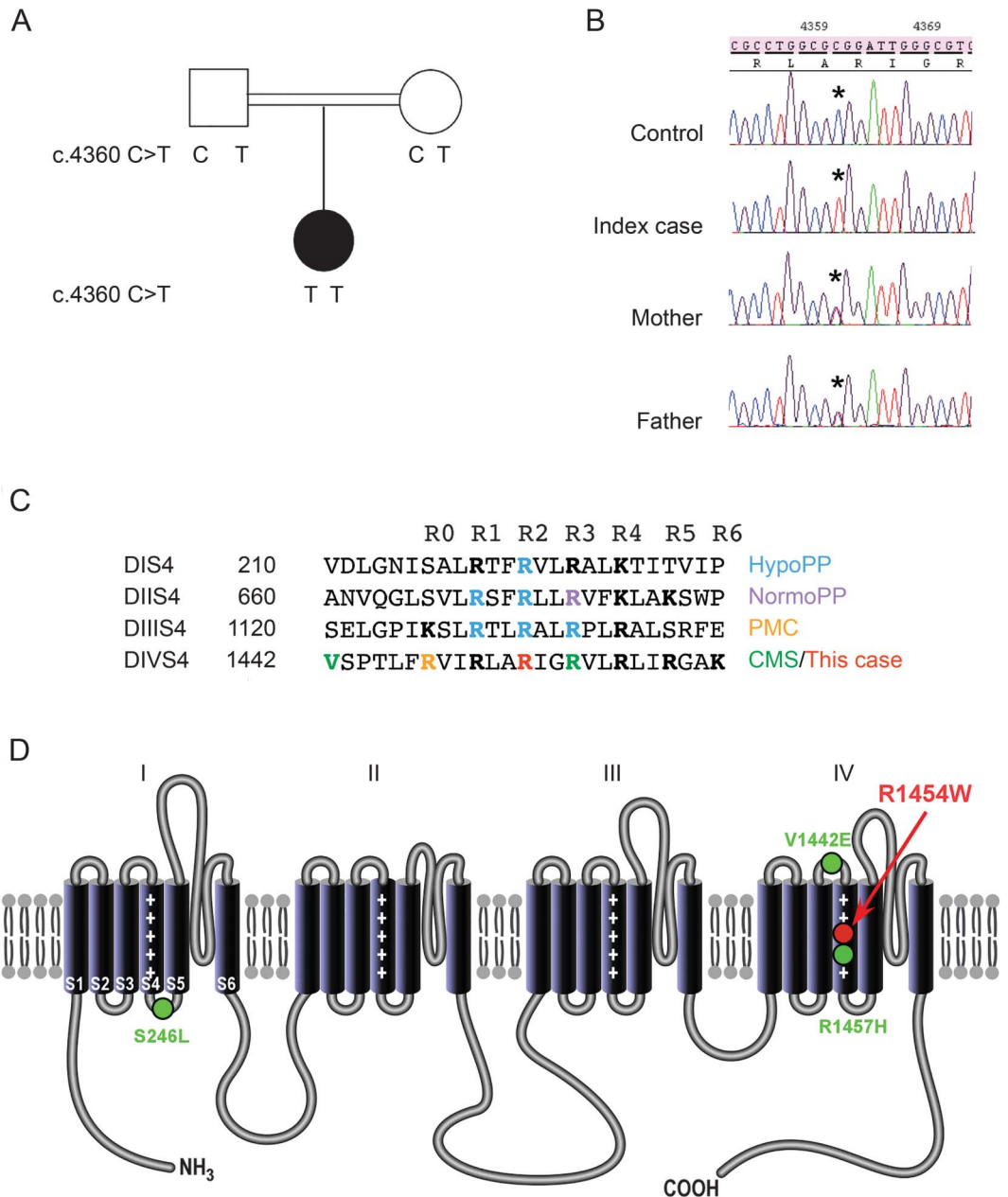
**RESULTS Clinical presentation of the patient.** The patient was a 26-year-old woman of Lebanese origin. Her 2 parents were first cousins, 4 of her sibs were healthy, and one 18-month-old sister had died of septic shock. The proband had global hypotonia with difficult sucking and swallowing during her first months of life, and had delayed postural and motor development with walking acquired at age 30 months. We first examined the patient at age 12.5 years. No mental retardation was present and scholarship was normal. She reported permanent but fluctuating fatigable muscle weakness with most severe attacks

leading sometimes to full paralysis with swallowing and respiratory difficulties. The attacks occurred 2–3 times a week and were several hours to several days long. Clinical examination suggested muscle weakness with inability to run and climb stairs, waddling gait, hyperlordosis, and a Gowers sign. We noticed diffuse muscle weakness affecting mostly limb girdles, proximal muscles, and neck and trunk flexion, as well as a fluctuating ptosis, hypomimia, and ophthalmoplegia. Percussion myotonia was absent, and deep tendon reflexes were normal. In the following years, fluctuating muscle weakness worsened, with bilateral facial palsy and difficult swallowing in addition to ptosis and ophthalmoplegia. At age 14 years, pyridostigmine treatment (up to 480 mg a day) was introduced but only partially benefited the patient with no effect on the attacks. Stabilization of symptoms was observed at age 17 years with a muscle myasthenic score ranging from 30 to 40/100.<sup>12</sup> When she was 23 years old, the patient reported hour-long episodes of nonpainful muscle contractures triggered by cold, suggesting sodium channelopathy. The serum potassium levels were always found in the normal range. Acetazolamide (250 mg per day) treatment associated with potassium uptake was prescribed, but no clinical improvement was observed. All treatment except potassium uptake (up to 4,500 mg a day) was progressively stopped at 23 years, and the patient noted a better quality of life. After a few months, she still presented paralytic attacks lasting 1 hour 4 to 5 times a day without any trigger.

We performed electroneuromyography (ENMG) investigations 6 times from age 13–26 years but they did not help to establish a clear diagnosis. We never observed myotonic discharges. No decremental responses to repetitive nerve stimulations at 3 Hz were recorded, which is not in favor of the diagnosis of CMS. Decremental muscle response equal to 27% (from 11.9 mV pre-exercise to 8.7 mV postexercise) was recorded 40 minutes after a long exercise at room temperature, which is below the significance retained to ascertain the diagnosis of PP (–40%).<sup>9,13</sup>

**The homozygous p.R1454W substitution in  $\text{Na}_v1.4$  is linked to the disease.** We first excluded the most frequent genes linked to CMS ( $\epsilon$ ,  $\alpha$ ,  $\beta$ , and  $\delta$  subunits of acetylcholine receptors, *CHAT*, *DOK7*, *rapsyn*, and *MuSK*) by Sanger sequencing. We then screened *SCN4A* and found a homozygous c.4360C>T (p.R1454W) transition in exon 24 (figure 1). It was not reported as a polymorphism in public databases, and we did not observe it in 200 chromosomes from Lebanese controls. The p.R1454W substitution affects the third arginine residue of DIVS4 and has never been reported to be substituted (figure 1). The clinical history and examination and neurophysiologic investigations

**Figure 1** SCN4A and p.R1454W missense substitution



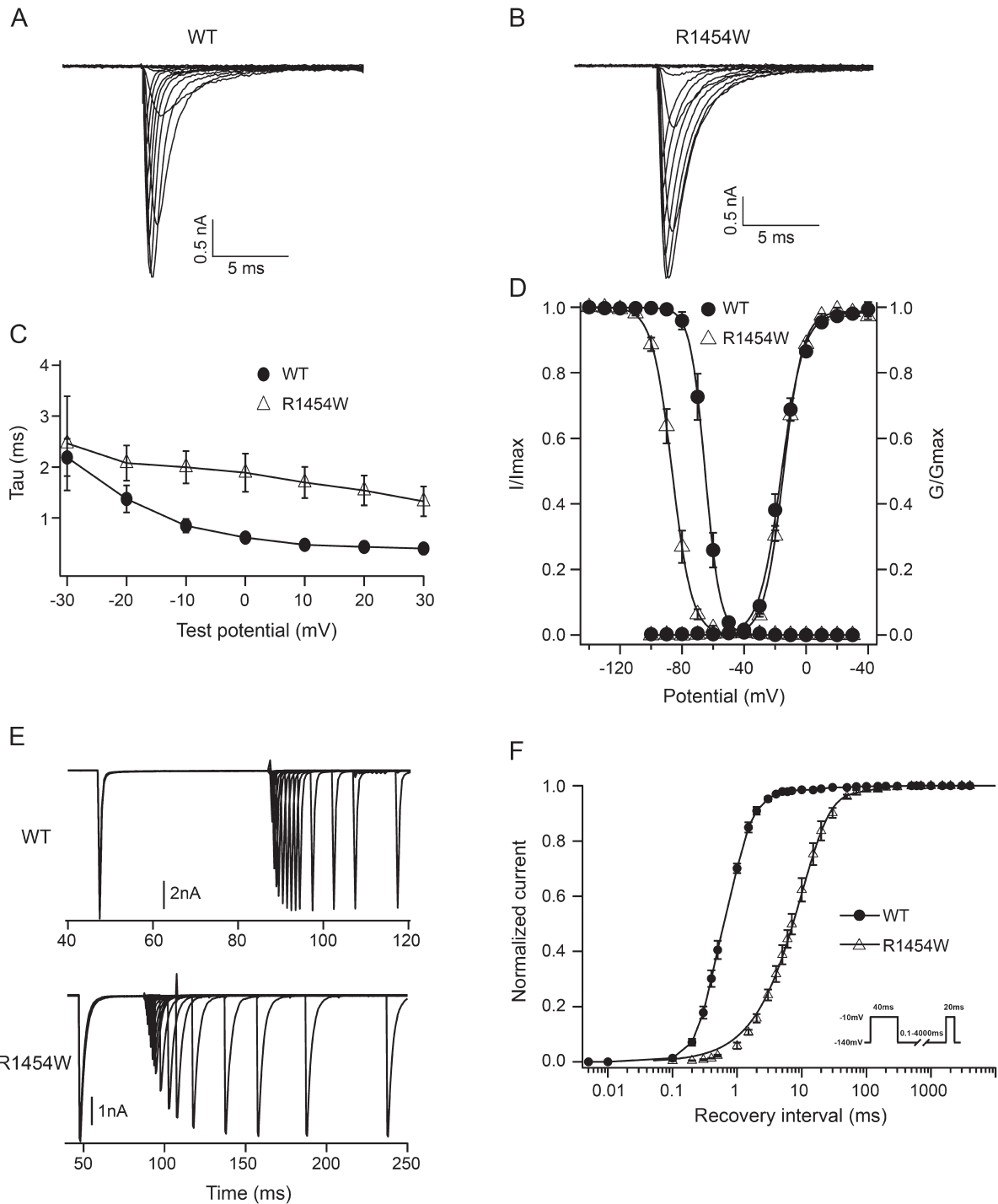
(A, B) Family tree and electropherogram of SCN4A sequence (part of exon 24). The 2 healthy and consanguineous parents are heterozygous for the c.4360C>T transition (asterisk in B), which is homozygous in the patient (index case). (C) Alignment of the S4 segments of the 4 domains (DI-DIV, human sequence), which contain charged residues at every third position numbered R0 to R6 (bold). The phenotype resulting from their substitution is indicated (hypokalemic periodic paralysis [HypoPP] in blue, normokalemic periodic paralysis [NormoPP] in purple, paramyotonia congenita [PMC] in orange, [CMS] in green). The p.R1454W substitution affects the R2 residue of DIVS4 (in red). (D) Cartoon of the pore-forming  $\alpha$  subunit of Na<sub>v</sub>1.4 and location of the substitutions linked to congenital myasthenic syndromes (CMS): p.S246L and p.V1442E were heteroallelic, whereas p.R1454W and p.R1457H were homozygous.

were normal for both heterozygous parents, supporting a recessive inheritance of the phenotype.

**p.R1454W channels have multiple defective biophysical properties.** We recorded sodium currents in tsA201 cells expressing WT or p.R1454W channels. The mutant channel current decayed more slowly than the WT over the voltage range tested (figure 2, A

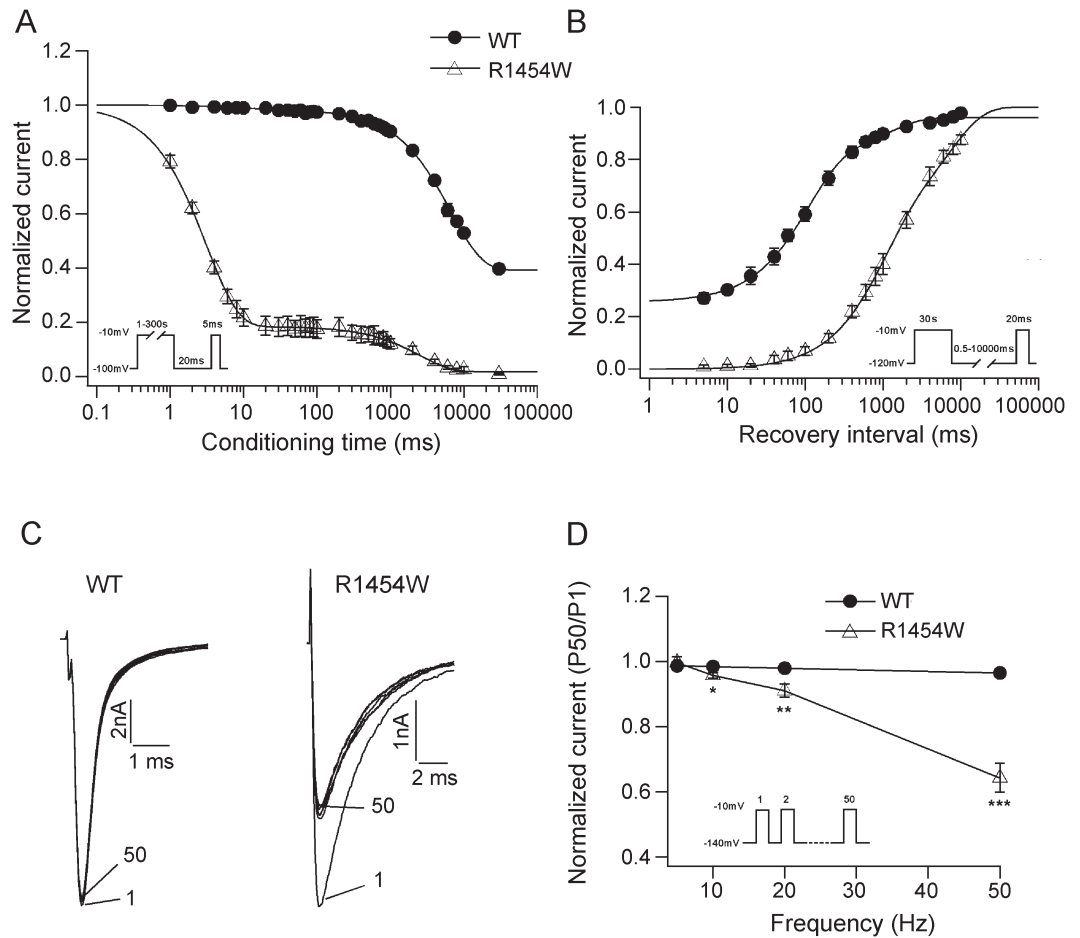
and B). Kinetics of fast inactivation were slowed for p.R1454W (n = 55) compared to the WT channel (n = 12): at 0 mV, the inactivation time constant of the mutant channel was approximately 4 times higher than that of the WT (p < 0.01, figure 2C). There was no change in the current density between the WT and the mutant channels (figure e-1). We also evaluated the deactivation properties of WT and mutant

**Figure 2** Functional consequences of the p.R1454W substitution on the Na<sup>+</sup> channel properties



(A, B) Na<sup>+</sup> current traces recorded in the whole-cell configuration for WT channels (A) and p.R1454W mutant channels (B). Cells were held at  $-100$  mV, then depolarized to a test pulse ranging from  $-100$  to  $+50$  mV in  $10$ -mV increments for  $50$  ms. (C) Time constants of fast inactivation. Fast inactivation phase decays for wild-type (WT) (filled circles,  $n = 12$ ) and p.R1454W (empty triangles,  $n = 55$ ) were fitted with a single-exponential function for voltages from  $-30$  to  $+30$  mV, for a  $-100$  mV holding potential. Each value represents the mean  $\pm$  SEM. (D) Steady-state activation and inactivation. For steady-state fast inactivation for WT ( $n = 10$ ) and p.R1454W ( $n = 8$ ) channels, cells were held at prepulse potentials from  $-100$  mV to  $+10$  mV for  $200$  ms, and were then subjected to a  $-10$  mV test pulse for  $5$  ms. For steady-state activation for WT ( $n = 12$ ) and p.R1454W ( $n = 31$ ) channels, peak Na<sup>+</sup> conductance ( $G_{Na^+}$ ) was measured during a  $50$ -ms test pulse to various test potentials from a holding voltage of  $-100$  mV.  $G_{Na^+}$  was calculated from  $G_{Na^+} = I_{Na^+} \times (E - E_{rev})$ , where  $I_{Na^+}$  is the peak Na<sup>+</sup> current during the test depolarization (E) and  $E_{rev}$  is the Na<sup>+</sup> current reversal potential. Each value represents the mean  $\pm$  SEM. (E) Current traces obtained in response to the 2-pulses protocol. The cells were depolarized to  $-10$  mV for  $40$  ms from a holding potential of  $-140$  mV to inactivate all the Na<sup>+</sup> channels. Test pulses were then applied to  $-10$  mV for  $20$  ms to measure current amplitudes, which represent the portion of channels that recover from inactivation, with an interval ranging from  $0.1$  to  $4,000$  ms. (F) The resulting curves were fitted to a monoexponential equation. The time constant of the mutant channel ( $10.80 \pm 1.32$  ms) was higher than the one of WT channel ( $0.99 \pm 0.06$  ms,  $p < 0.001$ ).

**Figure 3** Slow inactivation and frequency-dependent regulation of Na<sup>+</sup> current



(A) Onset of slow inactivation. The entry into the slow inactivation state was measured using a double-pulse protocol. The cells were first depolarized to  $-10$  mV for a variable duration (1–300 seconds) from a holding potential of  $-100$  mV. Following an interpulse of 20 ms at  $-100$  mV to allow complete recovery from fast inactivation, a brief test pulse of 5 ms at  $-10$  mV was applied to measure the Na<sup>+</sup> current (fraction of channels not inactivated). The resulting curves were fitted with a double exponential equation, which yielded 2 time constants:  $\tau_{fast}$  and  $\tau_{slow}$ . The rate of entry to slow inactivation was strongly accelerated by p.R1454W. The  $\tau_{fast}$  of mutant channels represents  $80.55 \pm 3.89\%$  of the  $\tau$  component, compared to  $2.58 \pm 0.64\%$  for wild-type (WT) channels. (B) Recovery from slow inactivation. Transfected cells were depolarized to  $-10$  mV for 30 seconds from a holding potential of  $-120$  mV to inactivate the Na<sup>+</sup> channels. Test pulses were then applied to  $-10$  mV for 20 ms to measure current amplitudes with an interval ranging from 0.5 ms to 10 seconds. After a long (30 seconds) conditioning pulse, the  $\tau_{fast}$  ( $803.73 \pm 161.55$  ms) and  $\tau_{slow}$  ( $3,091.20 \pm 525.70$  ms) of mutant channels were strongly slowed compared to the  $\tau_{fast}$  ( $96.97 \pm 9.02$  ms) and  $\tau_{slow}$  ( $786.96 \pm 145.37$  ms) of WT channels. (C) Superposition of the Na<sup>+</sup> current traces obtained in response to a 50-pulse train applied at  $-10$  mV for 10 ms at a frequency equal to 50 Hz. (D) Relative Na<sup>+</sup> current amplitudes ( $P_{50}/P_1$ ) of the 50th sweep recorded after a frequency-dependent inhibition protocol. The currents generated by mutant channels were reduced by 2.8%, 6.9%, and 32.3% compared to the ones generated by WT channels at a frequency of 10, 20, and 50 Hz, respectively (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ;  $n = 5$ ).

channels by measuring tail current recorded at potentials ranging from  $-100$  mV to  $-60$  mV after activating the channels at  $+40$  mV for 0.5 ms. There was no difference between WT and mutant traces and time constant ( $p > 0.05$ , figure e-1).

The conductance-voltage relationship showed no difference between the WT and the mutant channel (figure 2D). The voltage for half-activation was not different between the mutant and the WT (WT:  $V_{0.5} = -15.9 \pm 3.7$  mV,  $n = 12$ ; p.R1454W:  $V_{0.5} = -14.6 \pm 3.4$  mV,

$n = 31$ ,  $p > 0.05$ ). By contrast, the voltage for half-inactivation was shifted toward more hyperpolarized potentials for the mutant compared to the WT channel (WT:  $V_{0.5} = -65.3 \pm 1.5$  mV,  $n = 10$ ; p.R1454W:  $V_{0.5} = -86.4 \pm 1.4$  mV,  $n = 8$ ,  $p < 0.001$ ; figure 2D). We assessed recovery from fast inactivation using a double-pulse protocol. The mutant channels exhibited a recovery from fast inactivation 10-fold slower than that of the WT channels (10.8 ms vs 0.99 for WT, figure 2, E and F,  $p < 0.001$ ).

Table Kinetics of slow inactivation		
	WT	R1454W
<b>Onset of slow inactivation</b>		
$\tau_{fast}$	16.23 ± 8.30	3.12 ± 0.34*
$A_{fast}$	2.58 ± 0.64	80.55 ± 3.89†
$\tau_{slow}$	6,224.4 ± 561.8	2,723.7 ± 375.8†
$A_{slow}$	63.25 ± 4.69	18.33 ± 3.98†
n	5	9
<b>Recovery from slow inactivation (4-s conditioning pulse)</b>		
$\tau_{fast}$	1.75 ± 0.16	74.72 ± 12.78†
$A_{fast}$	66.58 ± 5.85	48.12 ± 3.01†
$\tau_{slow}$	172.35 ± 23.94	2,918.16 ± 502.03†
$A_{slow}$	33.42 ± 5.85	51.88 ± 3.01†
n	4	7
<b>Recovery from slow inactivation (30-s conditioning pulse)</b>		
$\tau_{fast}$	96.97 ± 9.02	803.73 ± 161.55†
$A_{fast}$	49.64 ± 3.66	40.08 ± 6.23
$\tau_{slow}$	786.96 ± 145.37	3,091.2 ± 525.7†
$A_{slow}$	20.18 ± 3.59	51.93 ± 5.05†
n	4	5
<b>Recovery from inactivation</b>		
$\tau$	0.99 ± 0.06	10.80 ± 1.32†
A	99.78 ± 0.11	99.31 ± 0.06
n	8	5

Abbreviations: A = fraction of  $\tau$  components (%); n = number of cells tested;  $\tau$  = time constant (ms).

Values are mean ± SEM.

Statistical significance of the difference between the wild-type and the p.R1454W channel: \* $p < 0.05$ , † $p < 0.001$ , ‡ $p < 0.01$ .

The p.R1454W channels have a hastened slow inactivation and a frequency-dependent regulation of  $Na^+$  current. We compared the onset of slow inactivation of WT and mutant channels using a double pulse protocol (figure 3A). Although this protocol does not allow us to completely separate fast from slow inactivation, the mutant channel seemed to dramatically enter to a slow inactivated state compared to the WT channel: the time constant of the fits was 16 ms for WT and 3 ms for mutant channels for  $\tau_{fast}$ , and was decreased from 6,224 ms (WT) to 2,723 ms (p.R1454W) for  $\tau_{slow}$  (table).

We also assessed recovery from slow inactivation using a double pulse protocol. By contrast to the recovery from fast inactivation, a double exponential equation was required to obtain an accurate fit with a 4-second and 30-second conditioning pulse and yielded 2 time constants ( $\tau_{fast}$  and  $\tau_{slow}$ ) (figure 3B and figure e-2). The  $\tau_{fast}$  had a relatively large weight (~66%) compared with the  $\tau_{slow}$  (~33%) for a

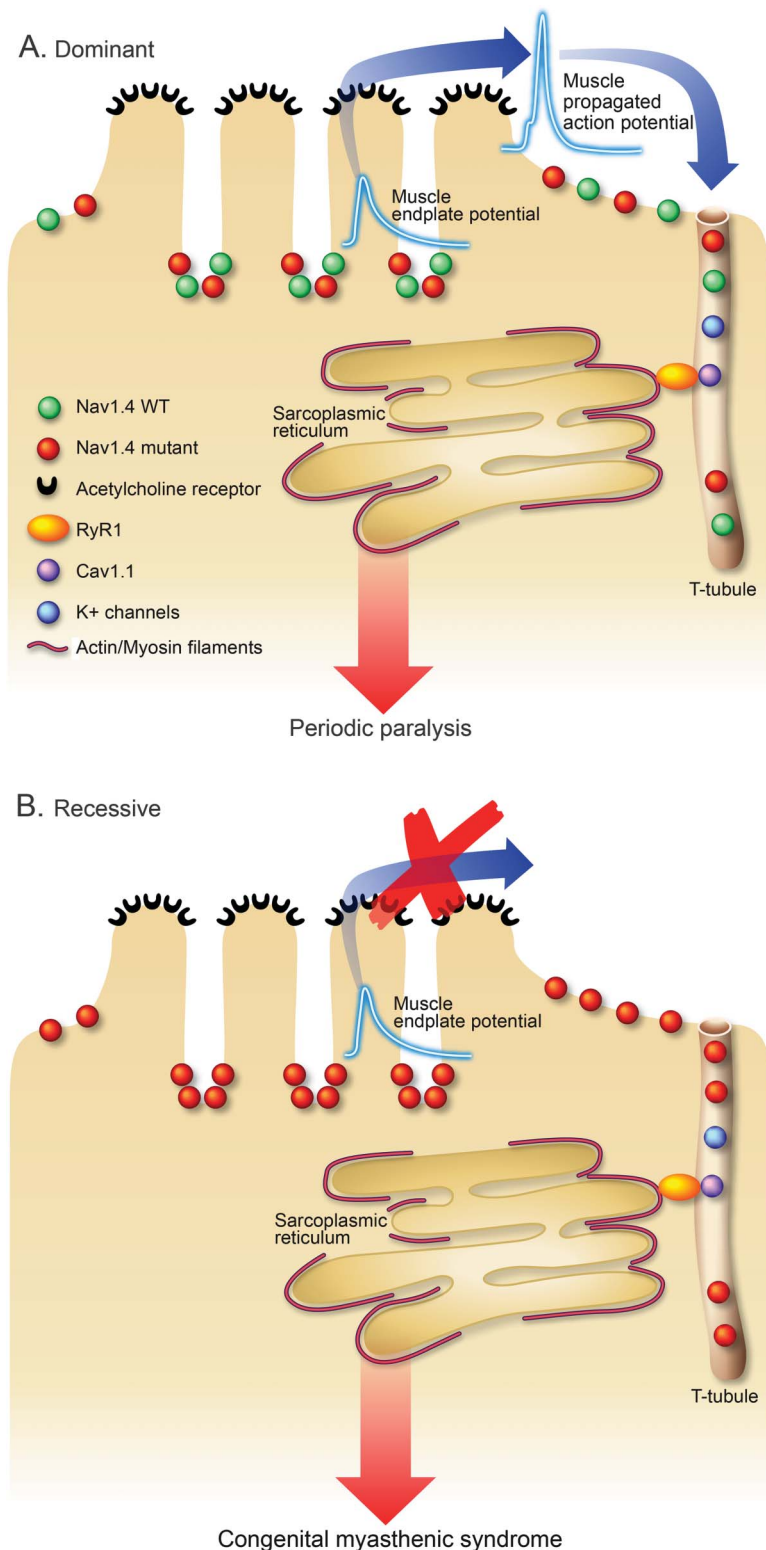
4-second conditioning pulse and 49% and 20% for a 30-second conditioning pulse. Indeed, the time constants of p.R1454W channels under the 2 conditioning pulses were slower compared to WT (table), indicating that p.R1454W slowed recovery from slow inactivation.

Finally, we assessed the effect of repetitive pulses on the  $Na^+$  current at a frequency of 5, 10, 20, and 50 Hz, a range that covers the known muscle firing rates. We observed a decrease in the current amplitude for mutant channels at a frequency of 10 Hz and above but not for the WT channels (figure 3, C and D). These data are consistent with the notion that the defective inactivation properties of the mutant channels may induce fatigable weakness during muscle firing.

**DISCUSSION** We report a patient with a recessive form of fatigable muscle weakness with most severe and reversible attacks that result from the homozygous p.R1454W mutation in *SCN4A*. The mutant channels displayed a combination of gating behavior defects that favor the inactivation state.

The clinical presentation of the patient is complex since it encompasses symptoms and signs of CMS and PP: (1) a permanent but fluctuating muscle weakness with ptosis and ophthalmoplegia, and transitory periods of improvement but no complete recovery that appeared early in life as observed in CMS; (2) reversible attacks of muscle weakness lasting from several hours to weeks, as observed in PP but not triggered by factors such as exercise, rest, potassium loading, or food. The absence of decremental response to repetitive nerve stimulations in ENMG investigations may challenge the diagnosis of CMS. However, it is sometimes not observed in patients with well-documented CMS, leading us to not exclude the hypothesis of CMS diagnosis.<sup>14</sup> In the 2 patients previously reported with  $Na_v1.4$  mutations, decrement was not observed in response to a 3-Hz nerve stimulation but to higher frequencies, which we did not test on the patient owing to pain.<sup>7,8</sup> Paramyotonia congenita was excluded by the absence of myotonia at percussion and needle EMG and ENMG as described by Fournier et al.<sup>9</sup> A trend towards reduced compound muscle action potential during the long exercise test was observed, prompting us to pursue the hypothesis of PP diagnosis.<sup>10</sup> However, most of the attacks occurred spontaneously, and ptosis or ophthalmoplegia are not usually observed in PP. We therefore propose that the patient displayed a mixed phenotype combining CMS with normokalemic PP. Different treatments were administered during the 15-year follow-up period but were not fully efficient. Carbonic anhydrase inhibitors (acetazolamide) and potassium uptake were given without benefit for the patient. This observation cannot be taken as an

**Figure 4** From periodic paralysis (PP) to congenital myasthenic syndromes (CMS)



The binding of acetylcholine to its cationic acetylcholine receptors at the neuromuscular junction (NMJ) leads to influx of  $\text{Na}^+$  within the muscle fiber, giving rise to the muscle endplate potential. The clustered  $\text{Na}_v1.4$  channels in the depths of the postsynaptic folds sense this small membrane depolarization and activate, thereby generating the muscle action potential (AP) at the NMJ. The large depolarization induced by the activation of  $\text{Na}_v1.4$  spread through the membrane up to the T-tubules to trigger muscle contraction through excitation-contraction coupling. In the dominantly inherited PP feature (A), there are 2

argument against the diagnosis of PP since some patients with PP and linked to  $\text{Na}_v1.4$  do not respond to this treatment.<sup>15</sup> The patient partially benefited from cholinesterase inhibitors (pyridostigmine), further suggesting that part of the muscle weakness resulted from defective neuromuscular transmission.

The S4 segments of  $\text{Na}_v1.4$  contain positively charged amino acids, which are critical for the voltage sensitivity of activation but are linked to distinct phenotypes when mutated.<sup>16</sup> The p.R1454W substitution is located between the recessive p.R1457H CMS-related mutation and the dominant p.R1448H/C mutation leading to paramyotonia congenita.<sup>17</sup> p.R1454W slowed current decay of fast inactivation as observed for some paramyotonia congenita-linked mutations.<sup>3,17–19</sup> The slow decay in the fast inactivation was accompanied with a delay in channel deactivation in the latter, and the combination of both defects may account for repetitive muscle AP. The channel deactivation was not affected by p.R1454W, which may account for its divergent effect on muscle excitability. Most of the substitutions affecting charged residues of S4 in DI, DII, and DIII of  $\text{Na}_v1.4$  and  $\text{Ca}_v1.1$  resulted in hypokalemic PP by improving a gating pore current.<sup>20–22</sup> We have previously demonstrated that DIVS4 of  $\text{Na}_v1.4$  has a distinct topology that required simultaneous substitutions of R0, R1, and R2 residues to promote this current.<sup>23–25</sup> Others have shown that R0 of DIVS4 does not promote gating pore current when mutated.<sup>23</sup> Nevertheless, we recorded gating pore current in *Xenopus* oocytes, and confirmed that p.R1454W has no effect, as expected from these studies (figure e-3).

The 3  $\text{Na}_v1.4$  mutations associated with a myasthenic phenotype are located in (p.R1454W and p.R1457H) or close to (p.V1442E) DIVS4. All led to hyperpolarization of the steady-state fast inactivation, slow recovery from inactivation, and reduced the channel ability to activate in response to repetitive stimulating pulses that may account for fatigable muscle weakness.<sup>6,7</sup> The slow inactivation was not severely affected by p.V1442E and p.R1457H, in

populations of  $\text{Na}_v1.4$  present at the postsynaptic cleft (wild-type [WT] and mutant) at high density. The WT fraction (representing 50%) is sufficient to generate AP that will spread along the sarcolemma up to the T-tubules. Abnormal gating behavior of the mutant fraction is proposed to result in sustained depolarization of the sarcolemma that will exert a dominant-negative effect on the WT fraction, leading hence to the inactivation of all  $\text{Na}_v1.4$  channels and no propagation of the muscle AP.<sup>4</sup> In the CMS-PP feature (B), all  $\text{Na}^+$  channels are defective and locked in an inactivated state, rendering the probability of AP initiation and propagation low. Postsynaptic or sarcolemma blocks occur with firing as a result of increased probability for the mutant channels to be inactivated, leading to permanent but fluctuating and acute attacks of muscle weakness, which are the clinical hallmarks of CMS and PP, respectively.

contrast to p.R1454W. Slow inactivation is a process distinct from fast inactivation that occurs in the order of seconds to minutes. It would be experimentally difficult to distinguish the contribution of fast and slow inactivation to the phenotype. A role of abnormal slow inactivation has already been proposed in nondystrophic myotonia and PP.<sup>26,27</sup> The p.R675Q substitution (DIIS4) linked to normokalemic PP was shown to enhance slow inactivation without affecting activation or fast inactivation.<sup>28</sup> Na<sub>v</sub>1.4 slow activation would play a prominent role in the regulation of muscle excitability, especially in fast twitch muscles that have the higher muscle firing properties.<sup>29</sup> We therefore suggest that both fast and slow inactivation defects account for the phenotype of muscle weakness in the patient described here.

The recessive inheritance of p.R1454W and its effect on Na<sub>v</sub>1.4 gating behavior are in accordance with a loss-of-function effect except for the modest slow current decay. Incomplete penetrance or a most severe phenotype due to homozygosity of a dominant mutation could be argued.<sup>30,31</sup> We do not favor this hypothesis since the biophysical defects of p.R1454W are in full accordance with a loss of function that does not impact the activity of the WT channel. Another possibility would be a reduced channel density at the membrane. We did not observe any reduced expression of the mutant channels by heterologous expression (figure e-1), and the p.R1454W mutation does not affect the sites known to be critical for Na<sub>v</sub>1.4 membrane stability, which is again not in favor of this second hypothesis.<sup>32,33</sup>

Altogether, our results led us to propose the following pathophysiologic hypothesis (figure 4). The location of Na<sub>v</sub>1.4 in the depths of postsynaptic folds and in the T-tubules favors an effective depolarization of the membrane. The high Na<sub>v</sub>1.4 density in these narrow spaces would compensate the defective inactivation of mutant channels when WT channels are present. When all channels are mutated, the probability of muscle AP initiation and propagation during firing would be reduced with increased probability of Na<sub>v</sub>1.4 inactivation, leading to fatigable muscle weakness. The defective activity of mutant Na<sub>v</sub>1.4 may not only block the genesis of muscle AP at the NMJ as observed in CMS, but also the muscle AP propagation or the excitation-contraction coupling. This may account for the overlap between the CMS and PP phenotype observed in the patient homozygous for p.R1454W. The expressiveness of the disease-causing mutation would vary with time for reasons that remain to be identified in nearly all sodium channelopathies of the central and neuromuscular systems.

#### AUTHOR CONTRIBUTIONS

K. Habbout: design, acquisition, analysis of the data, and drafting of the manuscript. Dr. Poulin: acquisition, analysis of the data, and drafting of the manuscript. Dr. Rivier: follow-up of the patients and drafting of the

manuscript. Dr. Giuliano: acquisition, analysis of the data, and drafting of the manuscript. Dr. Sternberg: acquisition of data and paper discussion. Dr. Fontaine: follow-up of the patients and drafting of the manuscript. Dr. Eymard: follow-up of the patients and drafting of the manuscript. Dr. Morales: follow-up of the patients and drafting of the manuscript. Dr. Echenne: follow-up of the patients and drafting of the manuscript. L. King: acquisition, analysis of the data, and drafting of the manuscript. Dr. Hanna: drafting of the manuscript. Dr. Männikkö: acquisition, analysis of the data, and drafting of the manuscript. Dr. Chahine: design, acquisition, analysis, interpretation of the data, and drafting of the manuscript. Dr. Nicole: design and conceptualization of the study, acquisition, analysis, interpretation of the data, and drafting of the manuscript. Dr. Bendahhou: design and conceptualization of the study, acquisition, analysis, interpretation of the data, and drafting of the manuscript.

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#### DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to [Neurology.org](http://Neurology.org) for full disclosures.

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