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## Homozygous C-terminal loss-of-function Na<sub>v</sub>1.4 variant in a patient with congenital myasthenic syndrome

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### Introduction

Congenital myasthenic syndromes (CMS) are a group of rare inherited disorders of neuromuscular transmission.<sup>1</sup> Clinical presentations range from predominant ptosis, ophthalmoparesis, facial and bulbar weakness, and generalized muscle weakness to predominant limb-girdle weakness with sparing of the eye and face muscles. Symptoms may appear during the neonatal period, late childhood, adolescence or even adulthood. Clinical

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presentation and response to treatment may be influenced by the underlying molecular mechanism.

Mutations in more than 30 genes have been identified as causing CMS.<sup>1</sup> The primary pathogenic mechanism is defective neuromuscular junction (NMJ) transmission but may include central nervous system and skeletal muscle involvement. Biallelic loss-of-function (LOF) genetic mutations in *SCN4A* encoding skeletal muscle sodium channel Na<sub>v</sub>1.4 are a rare cause of CMS.<sup>2-6</sup> Heterozygous carriers are asymptomatic, demonstrating recessive inheritance. Na<sub>v</sub>1.4 conducts the depolarizing current of the skeletal muscle action potential that when reduced results in attenuated action potentials and muscle force.

Biallelic *SCN4A* LOF mutations can also be found in patients diagnosed with congenital myopathy<sup>6-8</sup> and occasionally hypokalemic periodic paralysis (hypoPP).<sup>9,10</sup> A common pathogenic mechanism can account for the notion that patients diagnosed with *SCN4A*-associated CMS may present with additional features of myopathy<sup>5</sup> or hypoPP<sup>4</sup>. The mutant Na<sub>v</sub>1.4 channels within *SCN4A* LOF clinical spectra show distinct functional defects. Mutations associated with congenital myopathy show a range of alterations on Na<sub>v</sub>1.4 channel function but one allele is often null.<sup>6-8</sup> Hitherto reported CMS-associated mutations enhance channel inactivation<sup>2-5</sup> and typically affect fourth voltage sensing domain (VSD) of Na<sub>v</sub>1.4, the key VSD implicated in control of channel inactivation. Depolarization of the muscle may lead to excess accumulation of the mutant channels in an inactivated state leading to episodes of muscle weakness. Mutant Na<sub>v</sub>1.4 channels associated with hypoPP typically show mixed loss- and gain-of-function (GOF) features, but dominant inheritance and depolarized muscle fibers in hypoPP patients indicate GOF features as the main pathogenic mechanism.<sup>6</sup> However, on occasion, hypoPP shows recessive inheritance suggesting a contribution of LOF features to the clinical presentation.<sup>9,10</sup>

## Results

We report a 25-year-old consanguineous Turkish woman who presented from infancy with fluctuating stridor, dysphonia, dyspnea and limb weakness that persisted for days or weeks and aggravated during menstruation. She gave informed consent for this study. Clinical examination showed mild hyperlordosis, dysphonia, and speech-induced stridor. Amyotrophy, fixed muscle weakness, palpebral ptosis, diplopia, dyskalemia or myotonia were not observed. Tendon reflexes, serum CK levels and serum potassium levels were normal during episodes. Nerve conduction studies, 3-Hertz repetitive nerve stimulations (RNS), post-exercise RNS, electromyography (EMG) were normal but long exercise test (LET) (supplementary methods) exemplified a 35% ulnar nerve CMAP decrease from baseline (53% from peak). Pulmonary function tests, electrocardiogram, echocardiogram, thymus CT-scan, and muscle biopsy were unremarkable. Anti-AchR & anti-MUSK serum antibodies were absent. Single-fiber EMG was not performed. As she presented with childhood-onset fluctuating muscle weakness predominantly involving bulbar muscles, she was eventually diagnosed with CMS. Pyridostigmine, amifanpridine, and acetazolamide treatments were unsuccessful.

Targeted exon sequencing (supplementary methods) found c.4949C>T variation in exon 24 of the *SCN4A* gene (NM\_000334.4) leading to a p.(Pro1650Leu) (P1650L) in C-terminus of Na<sub>v</sub>1.4 while *CHRNE* was normal. The mutated proline is conserved among NaV isoforms (Fig 1A) and the mutation is extremely rare in the gnomAD database (4/277124 alleles). The variation was homozygous in the patient but heterozygous in her asymptomatic parents and two brothers suggesting recessive inheritance.

Patch clamp analysis (supplementary methods) of wild-type and P1650L mutant channels did not reveal changes in the voltage dependence of activation, but the current density of the cells expressing the mutant channels was less than 30% of the cells expressing the wild-type channel (p<0.01, Fig 1). Voltage dependence of fast- or slow inactivation, or time constants of onset of open- or closed state inactivation or of recovery from inactivation did not differ between mutant and wild-type channels (Fig 1). These data indicate that P1650L mutation reduces functional expression levels of Na<sub>v</sub>1.4 channel.

## Discussion

Our patient was clinically diagnosed with CMS based on the presentation of fluctuating weakness predominantly affecting bulbar muscles. The genetic diagnosis of biallelic LOF *SCN4A* variants places the patient on a clinical spectra of congenital myopathy-CMS-hypoPP. Fluctuating weakness suggests a myasthenic rather than myopathic disorder. The very early onset of symptoms and absence of dyskalemia although not excluding a diagnosis of hypoPP are atypical. Although CMS best describes the clinical picture EMG investigations did not reveal indications for NMJ dysfunction. EMG signs can be negative in myasthenic disorders and are variable or absent in other *SCN4A* CMS cases.<sup>2-5</sup> The prolonged episodes of weakness can also be a feature of PP however, and the LET was positive with a CMAP profile reminiscent of that described for a hypoPP patient with homozygous Na<sub>v</sub>1.4 mutation.<sup>11</sup> Similar features of CMS with overlapping EMG evidence of hypoPP has been reported previously in a patient with biallelic LOF Na<sub>v</sub>1.4 variant.<sup>4</sup>

Unlike Na<sub>v</sub>1.4 variants associated with episodic weakness in other cases of CMS and hypoPP, P1650L reduced functional expression of the channel without gain- or loss-of function changes in biophysical properties of the channel. This suggests that biallelic Na<sub>v</sub>1.4 variants with reduced baseline availability without changes in channel biophysical profile can underlie episodic weakness. Reduced baseline availability of functional Na<sub>v</sub>1.4 channels likely increases the susceptibility of the muscle force to depolarization-induced channel inactivation.<sup>9</sup> Accordingly, heterozygous Na<sub>v</sub>1.4 knock-out mice present with latent myasthenia.<sup>12</sup>

Mutation P1650L affects the intracellular C-terminus rather than transmembrane VSDs as described for other CMS-associated *SCN4A* mutations. This suggests that mutations outside the transmembrane regions should also be considered when searching for genetic cause of CMS. P1650 is located in an EF hand-like motif that was recently shown to be important in the control of trafficking of other NaV channel isoforms.<sup>13</sup> This suggests attenuated trafficking as a potential mechanism for reduced functional expression of P1650L channel although this needs to be experimentally confirmed.

We conclude that reduced functional expression of Na<sub>v</sub>1.4 channel without changes in its biophysical properties may underlie episodic weakness and present clinically with an overlap of CMS and hypoPP.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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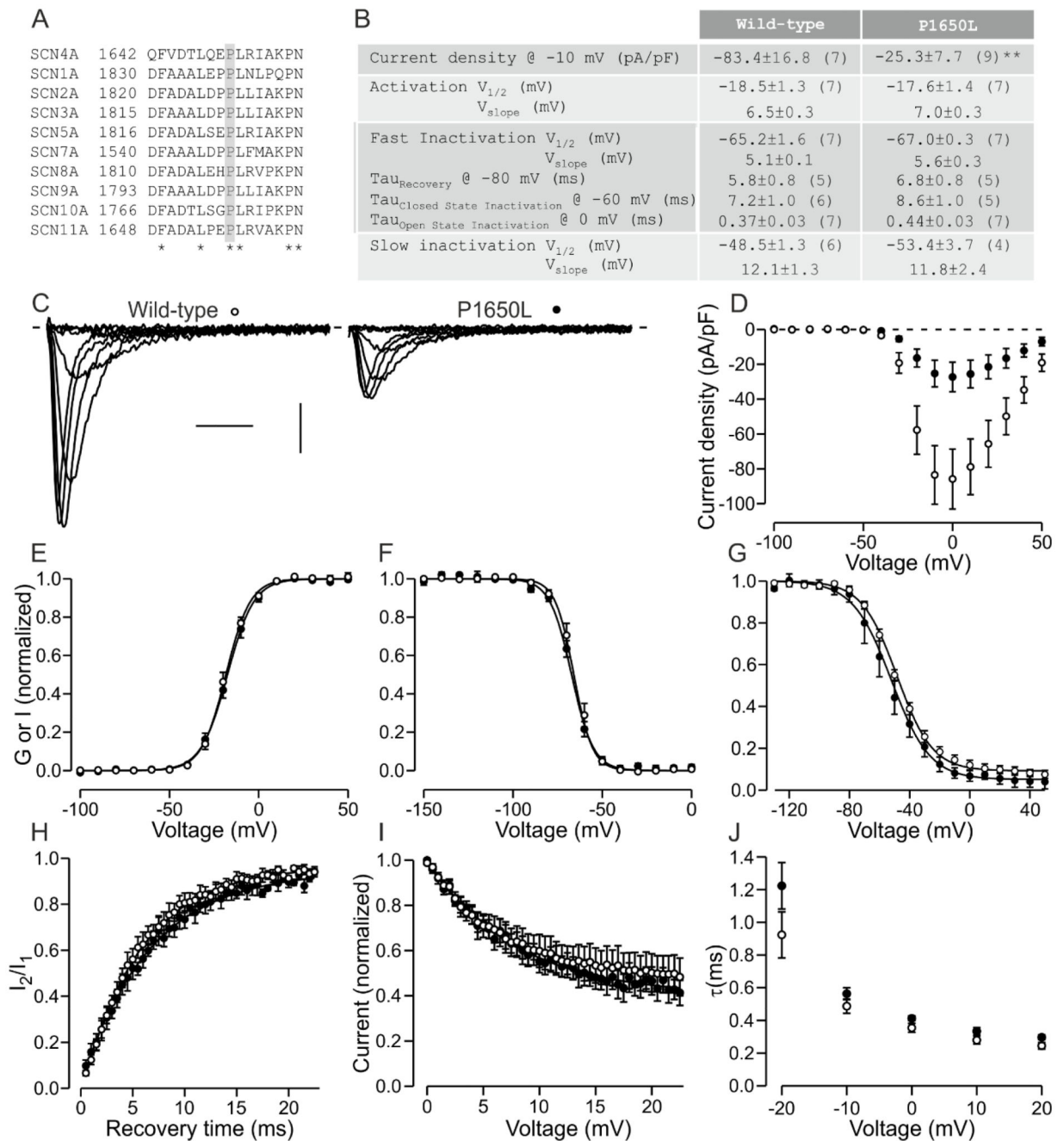
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**Figure 1.**

Functional characterization of the P1650L variant. A. Sequence alignment of Nav family.

Fully conserved proline is highlighted in grey. Fully conserved residues are indicated by \*.

B. Table of parameters for wild-type and P1650L channels. Number of cells is shown in parenthesis for each parameter. \*\*:  $p < 0.01$ .

C. Representative traces of wild-type and P1650L activation in response to voltage steps ranging from -60 mV to +10 mV. X-axis: 2

ms, y-axis: 20 pA/pF. D-J. Wild-type data is shown in open symbols, P1650L data in closed symbols. Solid lines show fit of Boltzmann (E-G) or exponential (H-I) equation to mean

recovery (H). J. Time constant of recovery vs voltage. Error bars represent SEM.

data. D. Current density is plotted against test voltage. E. Voltage dependence of activation. Peak conductance is plotted against test voltage. F-G. Voltage dependence of fast (F) and slow (G) inactivation. Current in response to test pulse is plotted against the pre-pulse voltage. H. Recovery from inactivation. Current in response to a second test pulse relative to current in response to first pulse is plotted against the duration at recovery voltage -80 mV. I. Onset of close state inactivation. Current in response to test pulse is plotted against duration of pre-pulse to -60 mV. J. Open state inactivation. Time constant of a fit of an exponential curve to inactivation following channel opening is plotted against the test voltage.