

MICRO REPORT

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Splice-variant specific effects of a *CACNA1H* mutation associated with writer's cramp



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Abstract

The *CACNA1H* gene encodes the $\alpha 1$ subunit of the low voltage-activated $\text{Ca}_v3.2$ T-type calcium channel, an important regulator of neuronal excitability. Alternative mRNA splicing can generate multiple channel variants with distinct biophysical properties and expression patterns. Two major splice variants, containing or lacking exon 26 (± 26) have been found in different human tissues. In this study, we report splice variant specific effects of a $\text{Ca}_v3.2$ mutation found in patients with autosomal dominant writer's cramp, a specific type of focal dystonia. We had previously reported that the R481C missense mutation caused a gain of function effect when expressed in $\text{Ca}_v3.2$ (+ 26) by accelerating its recovery from inactivation. Here, we show that when the mutation is expressed in the short variant of the channel ($- 26$), we observe a significant increase in current density when compared to wild-type $\text{Ca}_v3.2$ ($- 26$) but the effect on the recovery from inactivation is lost. Our data add to growing evidence that the functional expression of calcium channel mutations depends on which splice variant is being examined.

Keywords: *CACNA1H*, Calcium channel, $\text{Ca}_v3.2$, Alternative splicing, Splice variant, Writer's cramp

The *CACNA1H* gene encodes the pore-forming $\alpha 1$ subunit of the $\text{Ca}_v3.2$ calcium channel isoform which, along with $\text{Ca}_v3.1$ (*CACNA1G*) and $\text{Ca}_v3.3$ (*CACNA1I*), form the low-voltage activated T-type calcium channel family. The three isoforms generate currents with distinct biophysical and pharmacological properties and help regulate neuronal excitability, hormone secretion and cardiac function. Alternative mRNA splicing and differential splice-variant expression patterns further enhance the functional diversity of T-type channels [1].

Inherited or de novo mutations found in $\text{Ca}_v3.2$ have been associated with numerous disorders, including epilepsy, primary aldosteronism, pain, autism and amyotrophic lateral sclerosis (for review see [2]). Many of these mutations have been characterized using heterologous expression systems and although a subset of these variants have been shown to cause significant biophysical

changes, some produce mild or no alterations of channel function. A few studies have pointed out that mutations may differentially affect the activity of different splice variants, which can partially explain the lack of effects seen in previous reports [3–6]. Another important consideration is that missense, silent and non-coding mutations that do not alter channel function can potentially contribute to disease by disturbing exonic splicing regulatory sites, thus affecting the normal expression of variants [7].

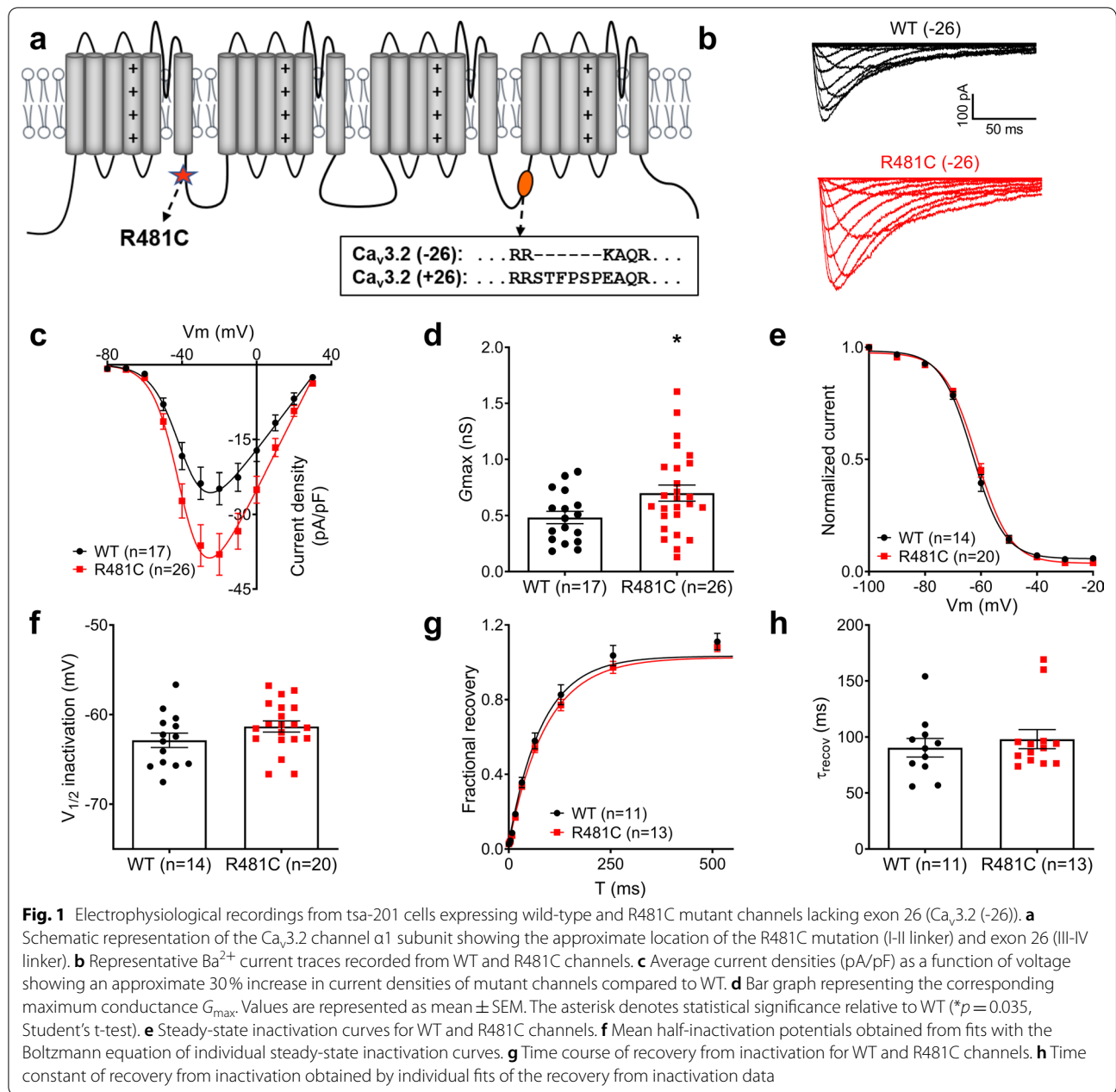
CACNA1H has been found to have at least 14 sites for alternative splicing with the potential to generate over 4,000 mRNA transcripts [7]. Two major splice variants, containing or lacking exon 26 ($\text{Ca}_v3.2$ (± 26)) have been found in multiple human tissues (corresponding to exon 25 in rat that is expressed in roughly half of the channel transcripts in the thalamus [3]). The inclusion of exon 26 adds 6 amino acids to the cytoplasmic domain III-IV linker region of $\text{Ca}_v3.2$ (Fig. 1a) and can cause changes in the biophysical properties of the channel, including a hyperpolarizing shift in the voltage dependence of activation and slower recovery from inactivation [7, 8]. We have recently shown that a R481C $\text{Ca}_v3.2$ missense

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variant that segregated in a family with autosomal dominant-inherited writer's cramp (WC) alters the biophysical properties of $Ca_v3.2$ (± 26) channels. Electrophysiological analysis of R481C cloned into $Ca_v3.2$ (+26) showed that mutated channels had a significant faster recovery from inactivation when compared to wild-type (WT) $Ca_v3.2$, while current density and steady-state inactivation properties remained the same [9]. Here, we tested the effects of this mutation in a channel backbone that lacks the exon 26 sequence in transfected tsa-201 cells. Figure 1b shows representative Ba^{2+} current traces from WT and

R481C channels. Different from what was seen in $Ca_v3.2$ (+26), when R481C was expressed in $Ca_v3.2$ (-26), we noted a significant increase in current density as shown in the current density-voltage relationship and maximal conductance graphs (Fig. 1c, d). Steady state inactivation and recovery from inactivation properties were not different from WT channels (Fig. 1e-h). It is important to note that while the R481C mutation causes different effects depending on which splice variant is being tested, these effects imply a $Ca_v3.2$ gain of function in both exon 26 containing and lacking channels, which can lead to

increased neuronal excitability and contribute to a dystonic phenotype [10]. Although unlikely, it is unknown whether patients carrying the R481C mutation may also have alterations in exon 26 splicing that may exacerbate the effect of the mutations in specific tissues.

As mentioned above, splice variant specific effects of point mutations have previously been shown for $Ca_v3.2$ channels. The R1584P mutation found in the genetic absence epilepsy rats from Strasbourg (GAERS) model only manifests itself functionally when introduced into the $Ca_v3.2$ variant that carries exon 26 (exon 25 in rats) [3]. Point mutations associated with primary aldosteronism have also been shown to have splice variant specific effects. Three mutations (S196L, V1951E and P2083L) caused significant changes in $Ca_v3.2$ (+26) but not in $Ca_v3.2$ (-26) channels, while M1549I altered both channel splice variants function [6]. Interestingly, the authors of this study found that human zona glomerulosa cells, which produce the hormone aldosterone, only express the long $Ca_v3.2$ (+26) channels. Since both splice variants are expressed in the brain, their results can partially explain why only patients with the M1549I mutation have neuronal abnormalities in addition to aldosteronism [6]. Splice variant specific effects of mutations have also been reported in the high-voltage activated $Ca_v2.1$ P/Q-type channels. Three type-1 familial hemiplegic migraine (FHM-1) mutations alter channel function differently when expressed in $Ca_v2.1$ containing or lacking exon 47 [4]. Our group has also reported an FHM-1 (Y1384C) mutation that has differential effects on recovery from inactivation dependent on which $Ca_v2.1$ splice variant (± 47) is being tested [5].

The R481C $Ca_v3.2$ mutation has been previously found in a patient with bilateral trigeminal neuralgia [11]. This raises the question as to why identical mutations can generate distinct phenotypes in different patients. In fact, highly penetrant mutations for severe Mendelian diseases have been found in healthy individuals [12]. Considering the number of mRNA transcripts that *CACNA1H* can generate, mutations can potentially produce different spatial and temporal effects depending on splice variant expression patterns. In addition, there is growing evidence suggesting the importance of other genes for the penetrance and expressivity of mutations [13]. Notably, besides the mutation in *CACNA1H*, three additional missense mutations in other genes segregated with disease phenotype in the family affected with writer's cramp [9]. One of these genes, *SPTBN5*, encodes the protein spectrin- βV , a member of the spectrin family of cytoskeletal proteins. Interestingly, our group has shown that $Ca_v3.2$ channels interact and can be modulated by at least three spectrin proteins: spectrin- αII , spectrin- βI and spectrin- βII [14]. Whether the mutation in the *SPTBN5*

gene affects the expression of the R481C mutation in *CACNA1H* contributing to the pathophysiology of WC is unknown. Finally, we note that splicing of exon 26 interferes with calnexin-dependent retention of the channel in the ER, thus increasing channel expression at the cell surface [15]. It is possible that in $Ca_v3.2$ (-26) channels, there is a synergistic effect between the domain I-II linker mutation and the enhanced ability of calnexin to facilitate ER export.

In conclusion, our study provides further evidence that *CACNA1H* alternative splicing may be important in the pathophysiology of genetic disorders and highlights complexity of the mechanisms by which a mutation can contribute to disease.

Abbreviations

RNA: Ribonucleic acid; mRNA: Messenger ribonucleic acid; WC: Writer's cramp; WT: Wild-type; GAERS: Genetic absence epilepsy rats from Strasbourg; FHM-1: Type-1 familial hemiplegic migraine.

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Authors' contributions

IAS co-designed the study, made the mutation, performed electrophysiological experiments and wrote the manuscript. MAG performed electrophysiological experiments. GWZ co-designed and supervised the study and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

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Consent for publication

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