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

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Abstract

The CACNA1H gene encodes the α1 subunit of the low voltage-activated Ca3.2 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 Ca3.2 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 Ca3.2 (+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 Ca3.2 (−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, Ca3.2, Alternative splicing, Splice variant, Writer’s cramp

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variant that segregated in a family with autosomal dominant-inherited writer’s cramp (WC) alters the biophysical properties of Cav3.2 (±26) channels. Electrophysiological analysis of R481C cloned into Cav3.2 (+26) showed that mutated channels had a significant faster recovery from inactivation when compared to wild-type (WT) Cav3.2, 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 Ba²⁺ current traces from WT and R481C channels. Different from what was seen in Cav3.2 (+26), when R481C was expressed in Cav3.2 (−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 Cav3.2 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,3.2 channels. The R1584P mutation found in the genetic absence epilepsy rats from Strasbourg (GAERS) model only manifests itself functionally when introduced into the Ca,3.2 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,3.2 (+26) but not in Ca,3.2 (-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,3.2 (+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,2.1 P/Q-type channels. Three type-1 familial hemiplegic migraine (FHM-1) mutations alter channel function differently when expressed in Ca,2.1 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,2.1 splice variant (±47) is being tested [5].

The R481C Ca,3.2 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,3.2 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,3.2 (-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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