Electrophysiological characterization of a Ca$_{v}$3.2 calcium channel missense variant associated with epilepsy and hearing loss

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Abstract

T-type calcium channelopathies encompass a group of human disorders either caused or exacerbated by mutations in the genes encoding different T-type calcium channels. Recently, a new heterozygous missense mutation in the CACNA1H gene that encodes the Ca$_{v}$3.2 T-type calcium channel was reported in a patient presenting with epilepsy and hearing loss—apparently the first CACNA1H mutation to be associated with a sensorineural hearing condition. This mutation leads to the substitution of an arginine at position 132 with a histidine (R132H) in the proximal extracellular end of the second transmembrane helix of Ca$_{v}$3.2. In this study, we report the electrophysiological characterization of this new variant using whole-cell patch clamp recordings in tsA-201 cells. Our data reveal minor gating alterations of the channel evidenced by a mild increase of the T-type current density and slower recovery from inactivation, as well as an enhanced sensitivity of the channel to external pH change. To what extent these biophysical changes and pH sensitivity alterations induced by the R132H mutation contribute to the observed pathogenicity remains an open question that will necessitate the analysis of additional CACNA1H variants associated with the same pathologies.

Keywords: Ion channels, Calcium channels, T-type channels, CACNA1H, Ca$_{v}$3.2, Mutation, Epilepsy, Hearing, Channelopathy

Mutations in the CACNA1H gene that encodes the Ca$_{v}$3.2 T-type calcium channel are risk factors for a number of human channelopathies including epilepsy [1], primary aldosteronism [2], autism spectrum disorder [3, 4], amyotrophic lateral sclerosis [5, 6], congenital amyotrophy [7], and trigeminal neuralgia [8, 9]. Recently, Algahtani and colleagues reported a new heterozygous missense mutation in a 50-year-old female patient with a clinical condition involving epilepsy and hearing loss which appears to be the first CACNA1H variant to be associated with sensorineural hearing alterations [10]. This mutation results in the substitution of an arginine at position 132 with a histidine (R132H) in the proximal extracellular end of the second transmembrane helix of Ca$_{v}$3.2 (Fig. 1a) and has not yet been reported in the gnomAD database (https://gnomad.broadinstitute.org/).
Molecular simulation using the AlphaFold-generated model of the human Ca₃.2 channel shows that replacement of the arginine 132 with a histidine leads to an additional hydrogen bond with methionine 119 of the first transmembrane helix (Fig. 1a) that has the potential to alter the gating of the channel. In addition, a histidine residue has a highly variable pKa value depending of its direct environment indicating that its charge may vary subtly as a function of external pH. To challenge this hypothesis, we assessed the functional impact of the R132H variant on the biophysical properties of Ca₃.2 using patch-clamp recordings in tsA-201 cells bathed in 5 mM barium as the charge carrier (see Additional file 1).

Both cells expressing Ca₃.2 wild-type (WT) and R132H mutant channels displayed characteristic low-voltage activated T-type currents (Fig. 1a, b). A significant 40% increase of the maximal macroscopic T-type conductance (Gₘₜₜ) was observed in cells expressing the R132H variant on the biophysical properties of Ca₃.2 (Fig. 1d) without any alteration of the voltage dependence of activation (Fig. 1e) or steady-state inactivation (Fig. 1f). An additional significant (p = 0.0342) slowing of the time constant (τ) of recovery from inactivation was observed for R132H channels (467 ± 21 ms, n = 18) compared to WT channels (284 ± 34 ms, n = 10) (Fig. 1g) while fast activation and inactivation kinetics of the current remained unaltered (Fig. 1h).

Next, we aimed to assess the effect of extracellular pH (pHₑₓ) on the regulation of the channels. Indeed, histidine residues theoretically bear a partial charge at physiological pH, although this is largely influenced by the direct environment of the residue, and therefore act as [H⁺] sensor as a result of protonation. Protonation may in turn mediate modulatory effects on voltage-gated channels, including Ca₃.2 [11]. Given that the R132H variant implicates the introduction of histidine within the extracellular end of the second transmembrane helix of Ca₃.2, we assessed the effects of extracellular pH changes, alkalinization (pHₑₓ 8.0) and acidification (pHₑₓ 6.5), on T-type currents. Consistent with previous results on T-type channels [11, 12], extracellular alkalinization and acidification produced a significant increase and decrease of the T-type current, respectively, in both Ca₃.2 WT- and R132H-expressing cells (Fig. 1i, top panels). However, these effects were emphasized on Ca₃.2 R132H-mediated currents. For instance, alkalinization-mediated increase of the T-type current was 82% higher (p = 0.0176) in cells expressing the R132H channel (24.0 ± 3.5% increase, n = 23) compared to cells expressing the WT channel (13.1 ± 1.2% increase, n = 16), whereas acidification-mediated decrease of the current was enhanced by 37% greater (p = 0.0087) (from −30.9 ± 2.9% decrease in WT, n = 15, to −42.5 ± 2.9% for R132H, n = 19) (Fig. 1i, bottom panels). In addition, extracellular alkalinization produced an acceleration of the kinetics of current activation and inactivation, whereas acidification produced the exact opposite (Fig. 1j, k, top panels). However, these effects were proportionally similar between WT and R132H channels (Fig. 1j, k, bottom panels).

Previous studies in animal models have documented the importance of T-type channels in the functioning of the auditory system. For instance, Ca₃.2 channels are highly expressed in mouse spiral ganglion neurons (SGN) where they are necessary for spatiotemporal auditory processing [13]. However, they also exhibit age-dependent increases in expression levels that are causally associated with SGN degeneration, whereas T-type channel
Fig. 1 (See legend on previous page.)
blocks are protective against age-related SGN and hearing loss [14]. Here, we showed that the Cav3.2 R132H mutation causes mixed alterations of the channel as evident from an increase in current density (that can be attributed to an alteration of the single channel gating properties and/or an increased expression of Cav3.2 at the cell surface) consistent with a gain-of-channel function. There is also a slowing of the recovery from inactivation which is consistent with a loss-of-function of the channel. However, the extent to which this loss-of-gating will manifest under physiological conditions will largely depend on the firing properties of nerve cells expressing the mutant channel. In addition, we illustrate that the R132H mutation enhances the impact of pH dependence of the channel. While the alterations may seem relatively mild, they have the merit to be observed and will require further experimentation to define their meaning in terms of pathogenicity. Clearly, there is evidence that alteration of pH homeostasis in response to primary metabolic disorders such as renal tubular acidosis is often accompanied by sensorineural hearing alterations [15]. In such context, altered pH-dependent modulation of Cav3.2 by the R132H mutation may represent a risk factor for hearing loss. Likewise, there is evidence that brain pH levels are significantly increased in experimental animal models of epilepsy [16–19] and patients [20] and precipitates the development of seizures. Therefore, it is a possibility that alkalinization-mediated increase of Cav3.2 R132H currents may also exacerbate seizures. An interesting consideration is whether a primary epilepsy could be the initiator of subsequent hearing loss in the patient carrying the Cav3.2 R132H mutation.

In conclusion, it is premature to recommend classifying the Cav3.2 R132H mutation as disease-causing variant at this stage in the absence of a larger number of variants causing the same pathologies. Moreover, since our functional analysis was performed in a recombinant expression system, there remains the possibility that the R132H mutation may exhibit a more pronounced phenotype in a native neuronal environment, and additional analysis will help to fully comprehend to which extent this mutation alters Cav3.2 channel function in the context of auditory function and epilepsy.

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Author contributions
RNS and LC performed the electrophysiology and analyzed the data. NW designed and supervised the study. NW wrote the manuscript. GWZ and MDW edited the manuscript. All authors critically revised the manuscript and contributed significantly to this work. All authors read and approved the final manuscript.

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Abbreviations
Cav Voltage-gated calcium channel
Gmax Maximal macroscopic conductance

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Additional file 1. Supplementary material and methods.
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