



CORRESPONDENCE

REVISED Mechanistic contribution of CaV3.2 calcium channels to trigeminal neuralgia pathophysiology not clarified [version 2; peer review: 2 approved]

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V2 First published: 29 Jun 2022, 11:718
<https://doi.org/10.12688/f1000research.122997.1>

Latest published: 27 Jul 2022, 11:718
<https://doi.org/10.12688/f1000research.122997.2>

Abstract

Trigeminal neuralgia (TN) is a rare, yet debilitating trigeminal pain disorder, with jolts of supramaximal-debilitating pain in one or more of the three trigeminal branches. Familial TN is now recognized, with a recent report describing several human genetic polymorphisms. One affected gene is the voltage-gated calcium channel, CaV3.2 (*CACNA1H*), with 19 polymorphisms first described. A recent study in PAIN by Gambeta-et-al (DOI:10.1097/j.pain.0000000000002651) is entitled "CaV3.2 calcium channels contribute to trigeminal neuralgia". Here, I call into question their claim. My main arguments are 1)-3): 1) Gambeta-et-al studied 4/19 mutations reported in heterologous cellular expression, with two mutations showing gain-of-function of CaV3.2, two mutations not showing gain-of-function. Therefore the exemplary picks of familial TN-associated CaV3.2 mutations do not show a uniform change of channel function, such as gain-of-function. 2) In Gambeta-et-al, one gain-of-function mutation, CaV3.2(G563R) was directed to mouse trigeminal ganglion (TG) neurons, and their resulting hyperexcitability was demonstrated. A critical control of a non-gain-of-function channel was not included here, it was unclear whether neurons were separated by sex, and human sensory neurons were not used. Importantly, it is not clear that TG neurons are the critical cellular site of CaV3.2 mutations. 3) Gambeta-et-al used CaV3.2-/- pan-null knockout mice. Human TN-associated CaV3.2 mutations were not over-expressed. They used an infraorbital nerve constriction injury and measured facial heat hyperalgesia. CaV3.2-/- show a pain phenotype similar to control, yet are not affected by a CaV3-inhibitory compound, Z944. My argument is that when starting with TN-associated human mutations, use of a trigeminal neuropathic pain model is of limited value, and that human mutations have to be expressed against a mouse null background. Re thermal cue, Gambeta-et-al failed to study cold-evoked pain which is a TN clinical hallmark.

Open Peer Review

Approval Status

	1	2
version 2 (revision) 27 Jul 2022	 view	 view
version 1 29 Jun 2022	 view	

- Nicholas A. Veldhuis** , Monash University, Parkville, Australia
Daniel P. Poole, Monash University, Parkville, Australia
- Kouichi Iwata**, Nihon University School of Dentistry, Tokyo, Japan

Any reports and responses or comments on the article can be found at the end of the article.

Thus, Gambeta-et-al's 2022 PAIN-paper offers little new mechanistic evidence why CaV3.2 polymorphisms are associated with trigeminal neuralgia.

Keywords

trigeminal neuralgia - CaV3.2 ion channel - channelopathy - pain - CACNA1H gene - familial trigeminal neuralgia

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Author roles: Liedtke W: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: WL is a full-time executive employee of Regeneron Pharmaceuticals Inc., Tarrytown NY, USA. WL co-founded TRPblue Inc. (Durham NC). The content of this Correspondence is not related to these situations. Beyond these, there is no other conflict of interest.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Liedtke W. **Mechanistic contribution of CaV3.2 calcium channels to trigeminal neuralgia pathophysiology not clarified [version 2; peer review: 2 approved]** F1000Research 2022, 11:718 <https://doi.org/10.12688/f1000research.122997.2>

First published: 29 Jun 2022, 11:718 <https://doi.org/10.12688/f1000research.122997.1>

REVISED Amendments from Version 1

This is a revised version vs originally submitted version, taking into account constructive and helpful criticism from Reviewer 1, three changes were implemented.

Any further responses from the reviewers can be found at the end of the article

In “*CaV3.2 calcium channels contribute to trigeminal neuralgia*” the authors state “*TN is a debilitating syndrome [...] Understanding the pathophysiology of TN is needed to provide new targets for therapeutic intervention.*” (Gambeta *et al.* 2022).—No disagreements here.

Whether their recent paper (Gambeta *et al.* 2022) indeed makes constructive progress on this front as claimed should be open for discussion. In this *Correspondence* I am asking for readers’ consideration whether data as reported and authors’ discussion of these data can indeed support their claim. In view of Dong *et al.* (2020), where polymorphisms in the *CACNA1H* gene were reported by Dong *et al.* as associated with familial trigeminal neuralgia, the lead question of “And what is new?” has to be asked, with particular focus on how findings by Gambeta *et al.* provide new mechanistic understanding of this debilitating pain condition in affected families. In other words, if a *CACNA1H* polymorphism carrier patient with trigeminal neuralgia asks their doctor “So tell me, doctor, why do I—and my family members—have these terrible jolts of pain, how does it really work?”; can they get a more rationally-based answer as a result of Gambeta *et al.*’s study?

Presented data appear the result of dedicated physiologic, animal behavior, neurocellular, molecular biology and biochemical experimentation. However, results as presented simply do not support the tendered claims and conclusions.

Gambeta *et al.* has three subsections, (i) directed expression of mutant CaV3.2 channels in heterologous cellular systems, (ii) directed expression of CaV3.2(G563R) in trigeminal ganglion sensory neurons, (iii) mouse studies using CaV3.2–/– pan-null knockout mice and a CaV3-selective chemical inhibitor, Z944. With regards to (i), there is non-congruence whether there is gain-of-function of the interrogated variants, or not. For (ii), the assumption that trigeminal ganglion sensory neurons are the critical cellular players in trigeminal neuralgia of CaV3.2 polymorphisms is unfounded. Only one variant is tested, not in human sensory neurons. With (iii), a model for trigeminal neuropathic pain after peripheral branch injury is used, and CaV3.2 mutations are not tested.

Thus, in terms of pathophysiology of trigeminal neuralgia we are left where we were before publication of Gambeta *et al.*’s recent paper in PAIN, perhaps scratching our heads more re the roles of CaV3.2 ion channels.

With regards to some critical detail:

The human genetic findings from Dong *et al.* (2020) are the starting point. As ion channels with some supporting evidence to their role in pathologic pain, CaV3.2 channels are legitimate targets for exploration of their mechanistic contribution to trigeminal neuralgia. How did the authors arrive at the four variants selected, out of a choice of 19? Ideally all variants should be interrogated by a validated first-pass screen. With an n=19 this is not asking too much, since previous studies that were based on interrogation of serially mutagenized channels and related signaling molecules have demonstrated feasibility (Nelson *et al.* 2010, Le *et al.* 2019, Kysilov *et al.* 2022). Any selection should be based on non-biased criteria, such as selecting polymorphisms from various subdomains of the molecule, representing equitably how the changes are distributed over the sequence (in case they do). The authors start with an arbitrary selection of mutations that then reveal channel function different from wildtype. Importantly, there is no unifying direction apparent in terms of the different-from-wildtype phenotype as presented. These data, taken in ensemble, indicate that the interrogated CaV3.2 channel mutations differ from wildtype, for each mutation in a different manner, with two of the mutations showing similar gain-of-function for peak current density. Importantly, the two other mutations do not reveal such a phenotype. It follows that lack of a specific explanatory electrophysiologic phenotype in half of interrogated channel mutations means that the detected gain-of-function in the other two CaV3.2 mutations is simply unclear with regard to their mechanistic impact on trigeminal neuralgia.

Then one of the CaV3.2 gain-of-function channels is directed for expression in adult mouse trigeminal ganglion sensory neurons, evoking hyperexcitability. However, the authors used mouse trigeminal ganglion sensory neurons, not human neurons, the latter a feasible experimental platform with stronger translational impact, especially for a paper with wider visibility, published in PAIN. The presented result is interesting, but lacking a critical control, namely at least one of the CaV3.2 mutations that did not reveal a gain of function. Importantly, how relevant is the examination of trigeminal ganglion sensory neurons with directed expression of CaV3.2(G563R)? How certain are we that trigeminal ganglion sensory neurons

are the critical cellular site for CaV3.2 mutations to contribute to trigeminal neuralgia? Findings in nerve injury, as presented here for infraorbital nerve, or as referenced for DRG-peripheral nerve injury, do not exclude other cellular lineages as critical sites of action for CaV3.2 mutations: glial cells could also be involved such as peripheral glia, namely Schwann cells (ganglionic and peripheral) and satellite cells, and central glia, namely astrocytes, microglia and even oligodendroglia. Besides trigeminal ganglion sensory neurons, there are trigeminal nucleus spinalis pain relay neurons where CaV3.2 is also expressed, possibly also in glial cells that co-function with these neurons. In which cell lineage CaV3.2 expression is critical is simply unknown in trigeminal neuralgia. Last but not least, trigeminal ganglion sensory neurons were derived from “5–7-week-old mice”—of which sex? Directed expression of a human trigeminal neuralgia-associated CaV3.2 mutation to trigeminal sensory neurons is at least as relevant for the experimental objective of Gambeta *et al.* as for their mouse *in-vivo* studies, yet for culture of trigeminal ganglion sensory neurons they were not conducting the experiments in a sex-separate manner as they did for mouse *in-vivo* experiments.

Now, onto the *in vivo* model. The authors have included several qualifier statements why infraorbital nerve constriction injury (CION) is not trigeminal neuralgia. These qualifiers are not wrong, but starting with human genetic polymorphisms that were detected in trigeminal neuralgia, NOT trigeminal neuropathic pain, dictates use of a more validated model, not of a marginally relevant model. Which outcomes of CION research have improved mechanistic understanding and clinical care of trigeminal neuralgia?

Can some cases of familial trigeminal neuralgia be reduced to a CaV3.2 channelopathy? If so, this would allow us to then translate from the infrequent genetic alteration toward the more frequent sporadic disease, sticking with the same target, CaV3.2. The animal data presented by Gambeta *et al.* do not pertain to this concept. They are disjointed because none of the human CaV3.2 mutations were introduced into the mouse, for a dedicated interrogation against a CaV3.2^{-/-} background. An experimental strategy of a *Cacna1h*^{-/-} mouse with directed expression of human CaV3.2 would have represented a logical follow up experiment - which was not part of Gambeta *et al.* As presented, there is simply a complete lack-of-connect between mouse data and human CaV3.2(mutant) physiology data.

Of the mouse data presented, the biggest red flag, peculiarly left unmentioned, is that CaV3.2^{-/-} pan-null mice show a highly similar nocifensive behavioral response to CION as do wildtype control mice! Non-injured pain behavior is less sensitive in CaV3.2^{-/-} mice, meaning there is a measurable effect on the trigeminal pain system, depending on presence/absence of CaV3.2. This finding can be interpreted as a “positive control” that the engineered CaV3.2 null mutation affects trigeminal pain processing for one of the measured parameters. But then the pain phenotype in terms of nocifensive behavior in CaV3.2^{-/-} is indistinguishable from wildtype in response to CION!

The documented different response to compound Z944—analgesic in wildtype for 30–60 min, complete lack of effect in CaV3.2^{-/-} mice—should prompt dedicated follow up studies to this interesting starting point, such as sensory neuron-specific knockdown of CaV3.2. But considering this result in the context of the other presented data, it follows that we do not understand trigeminal neuralgia pathophysiology better, and arguably we are also left confused in terms of contributions of CaV3.2 channels to trigeminal peripheral nerve constriction injury.

Moreover, the authors present hypersensitivity caused by CION as measured by heat avoidance. Clinically, in the context of trigeminal nerve pain, cold pain, *e.g.* as can be evoked by the slightest drafts of (cold) air, is a striking hallmark of the human disease. Inclusion of cold sensory cues, applied to trigeminally-innervated structures to evoke pain behavior, would have improved the study by increasing its reverse-translational impact.

Data availability

Underlying data

There are no data associated with this article.

References

- Dong W, Jin SC, Allocco A, *et al.*: **Exome Sequencing Implicates Impaired GABA Signaling and Neuronal Ion Transport in Trigeminal Neuralgia.** *iScience.* 2020; **23**(10): 101552.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gambeta E, Gandini MA, Souza IA, *et al.*: **CaV3.2 calcium channels contribute to trigeminal neuralgia.** *Pain.* 2022.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kysilov B, Hrcka Krausova B, Vyklicky V, *et al.*: **Pregnane-based steroids are novel positive NMDA receptor modulators that may compensate for the effect of loss-of-function disease-associated GRIN mutations.** *Br J Pharmacol.* 2022; **179**(15): 3970–3990.
- Le T, Jia Z, Le SC, *et al.*: **An inner activation gate controls TMEM16F phospholipid scrambling.** *Nat Commun.* 2019; **10**(1): 1846.
- Nelson O, Supnet C, Liu H, *et al.*: **Familial Alzheimer's disease mutations in presenilins: effects on endoplasmic reticulum calcium homeostasis and correlation with clinical phenotypes.** *J Alzheimers Dis.* 2010; **21**(3): 781–793.

Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 12 September 2022

<https://doi.org/10.5256/f1000research.136661.r150066>

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The CaV3.2 is the T-type calcium channel expressed in the brain's various neurons and glial cells and involved in the modulation of neuronal excitability and glial cell activation. The CaV3.2 is constructed from multiple subtypes, and these subtypes are involved in different functions in the brain. The CaV3.2 is also engaged in the alteration of nociceptive neuron activity associated with peripheral nerve injury or tissue inflammation. These observations strongly suggest that the CaV3.2 calcium channel contributes to the persistent pain occurrence. The current literature addressed the involvement of the CaV3.2 calcium channel in the trigeminal neuropathic pain mechanism. This literature is exciting and shows us many important issues regarding CaV3.2 in trigeminal persistent pain mechanisms.

I have just two suggestions in this literature: 1) The CaV3.2 channel is known to interact with various molecules, channels, and receptors. It's better to state these interactions. 2) If possible, it's better to address the schematic illustration showing the function of CaV3.2 in trigeminal neuropathic pain.

I do not have any concerns about indexing this article.

Is the rationale for commenting on the previous publication clearly described?

Yes

Are any opinions stated well-argued, clear and cogent?

Yes

Are arguments sufficiently supported by evidence from the published literature or by new data and results?

Yes

Is the conclusion balanced and justified on the basis of the presented arguments?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 September 2022

<https://doi.org/10.5256/f1000research.136661.r145594>

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Prof Liedtke has appropriately addressed our concerns and suggestions. We have no further comments and believe this is a well-written and well-justified correspondence that we hope will influence and inspire pain researchers carrying out similar studies in future.

Is the rationale for commenting on the previous publication clearly described?

Yes

Are any opinions stated well-argued, clear and cogent?

Yes

Are arguments sufficiently supported by evidence from the published literature or by new data and results?

Yes

Is the conclusion balanced and justified on the basis of the presented arguments?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: We each have >12 years experience in analgesic drug discovery utilising a range of pre-clinical pain models including migraine

We confirm that we have read this submission and believe that we have an appropriate level

of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 21 July 2022

<https://doi.org/10.5256/f1000research.135056.r142603>

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This correspondence provides a perspective on a recent publication by Gambeta et al, in the journal PAIN, entitled “CaV3.2 calcium channels contribute to trigeminal neuralgia”.

Overall, the critique captures and distills all of the key findings made by Gambeta et al. and relates them to a prior human study where polymorphisms in the CACNA1H gene were reported. By doing so it asks readers to consider “what is new” and whether findings in this study progress our mechanistic understanding of how CACNA1H polymorphisms contribute to trigeminal neuralgia.

The author raises pertinent and valid concerns, within the context of the clinical relevance, which no doubt has limited the mechanistic insight and translational impact of the study. This includes choice of the most appropriate and clinically relevant pain model, rationale behind selection of which missense mutations to assess (i.e. selected sites in only a single domain), sex-specific differences, the methods to assess function (e.g. comparing gain and loss of function on a null background, or lack of a cold pain model) and failure to consider the potential contribution of CaV3.2 expressed by other cell types that drive pain.

However, recognizing the expectations of a leading specialized journal such as PAIN, and the formative nature of this work, we suggest it would be useful to moderate some of these arguments and provide further constructive feedback, to benefit the authors of this specific study and for future consideration by others attempting related studies:

1. The comment about justifying the choice of these 4 particular mutants makes sense, but we suggest that author should equally justify screening all 19 mutants by pointing to any related studies in PAIN or equivalent calibre journals where a similarly comprehensive approach has been taken;
2. Comments about the lack-of-connect between mouse data and the human CaV3.2

physiology data would be nicely supported by suggesting logical follow up experiments. This may include assessment using a mouse model expressing the mutant human CaV3.2 or WT human channel, and/or determining specific roles through neuronal vs global deletion.

3. Final paragraph: Rather than stating that the authors simply did not consider the need to comment about the deficiencies of the study with respect to cold pain models, we suggest appending some constructive comments to this paragraph about how the study could have been improved if this had been added.

Is the rationale for commenting on the previous publication clearly described?

Yes

Are any opinions stated well-argued, clear and cogent?

Yes

Are arguments sufficiently supported by evidence from the published literature or by new data and results?

Partly

Is the conclusion balanced and justified on the basis of the presented arguments?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: We each have >12 years experience in analgesic drug discovery utilising a range of pre-clinical pain models including migraine

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 24 Jul 2022

Wolfgang Liedtke, New York University College of Dentistry, New York, USA

I am grateful to the reviewer for their careful appreciation of my comment, for their highly informed and constructive feedback. The reviewer's suggestions have been very helpful for revision of the ms.

Specifically, in response:

1. *"The comment about justifying the choice of these 4 particular mutants makes sense, but we suggest that author should equally justify screening all 19 mutants by pointing to any related studies in PAIN or equivalent calibre journals where a similarly comprehensive approach has been taken;"*

Three exemplary studies are now referenced.

2. *"Comments about the lack-of-connect between mouse data and the human CaV3.2*

physiology data would be nicely supported by suggesting logical follow up experiments. This may include assessment using a mouse model expressing the mutant human CaV3.2 or WT human channel, and/or determining specific roles through neuronal vs global deletion."

The logical follow up experiments are now explicitly spelled out, as suggested.

3. *"Final paragraph: Rather than stating that the authors simply did not consider the need to comment about the deficiencies of the study with respect to cold pain models, we suggest appending some constructive comments to this paragraph about how the study could have been improved if this had been added."*

Constructive comments, as suggested by the reviewer, are now appended to this paragraph.

Competing Interests: as indicated in the article, no change from CoI statement of the Correspondence.

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