A Critique of "A Gain Of-Function Mutation in $Na_V1.6$ in a Case of Trigeminal Neuralgia" by Tanaka et al. (2016)

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In "A Gain-Of-Function Mutation in $Na_V1.6$ in a Case of Trigeminal Neuralgia", Tanaka hypothesizes that chronic pain in Trigeminal Neuralgia (TN) can be, in the case of the study subject, caused by a gain-of-function mutation in the sodium channel $Na_V1.6$, where methionine-136 is substituted with valine in the domain I transmembrane segment 1 of the polypeptide. A Met136Val mutant is hypothesized to be hyperexcitable, increasing the number of high-firing neurons with high density currents and low current thresholds thus leading to the firing of more frequent and numerous action potentials. Rationally, these action potentials cause vascular compression of the root of cranial nerve V, especially in older patients where the arteries meet the root nerve V as it leaves the pons (Childs et al., 2000). When this neurovascular compression (NVC) occurs on the trigeminal nerve, it can lead to unilateral lancinating facial pain, a consistent symptom of TN. However, Tanaka mentions that there have been reported cases of TN without NVC, usually in younger patients, and that pain can occur in the absence of nerve compression. These caveats of the theory that NVC causes TN suggest to Tanaka that both genetic and epigenetic factors contribute to the pathophysiology of TN.

In Figure 2A and 2B, the current traces of sodium currents were recorded for ND7/23 cells in both wild-type (2A) and mutant (2B) channels. The results showed that the Met136Val mutant channel (2B) had a significantly larger current amplitude than the wild-type channel (2A), demonstrating that Met136Val mutants are more responsive to the same stimulus. This

hypersensitivity contributes to NVC and supports the hypothesis, as Met136Val mutants fire more numerous action potentials due to this larger current amplitude. In Figure 2C, the experiment was performed to observe the relationship between peak current and voltage for wild-type and Met136Val mutant channels. An increased peak current density was observed in Met136Val mutants compared to the wild-type channel, meaning that in a Met136Val mutant there is more current per cross sectional area of the neuron. Moreover, the increased (transient) peak current density contributes to the lower current threshold, indicating hypersensitivity. This hypersensitivity supports the hypothesis because the neuron is able to send more action potentials due to the increased peak current density. In Figure 2D, a comparison of voltage-dependent activation and steady-state fast inactivation was done in wild-type and Met136Val mutant channels, and in Figure E steady-state slow inactivation was measured. It was observed that the Met136Val mutation did not significantly affect the voltage-dependence of activation, fast inactivation, or slow inactivation. This indicates that the Met136Val mutation does not alter the intrinsic voltage-gating properties of the sodium channel, in the sense that the voltage threshold of the channel remains unchanged.

In Figure 3, the experiment was done to determine the resurgent sodium currents and average peak amplitudes. According to Lewis and Raman, resurgent currents result from a specific type of Na channel gating, in which the action potentials are unlikely to accumulate in fast inactivated states, “thereby shortening refractory periods and permitting rapid, repetitive, and/or burst firing” (Lewis and Raman, 2016). Resurgent currents were recorded from $Na_V1.8$-null TRG neurons expressing wild-type (3A) and Met136Val mutant (3B) channels. It is illustrated that the Met136Val mutants had larger resurgent currents than the wild-type

channels. Lewis and Raman's explanation of resurgent currents reveals that Met136Val mutant channels have shorter refractory periods and are thus more likely to exhibit rapid, repetitive, and/or burst firing patterns. These patterns occur because less time is required for a Met136Val mutant neuron to "recover" before being able to fire a subsequent action potential. The mutant channel's resurgent current properties explain the high frequency and number of action potentials associated with NVC. In Figure 3C, average peak amplitudes of resurgent currents were recorded in wild type and Met136Val mutant channel. The results in Figure 3C show that the average peak amplitude of the Met136Val mutant was 1.6x that of wild-type channels. Furthermore, the peak resurgent current density in Met136Val mutant channels was also significantly increased, contributing to lowering current threshold and increased excitability. Therefore, all of the data presented in Figure 3 is consistent with the hypothesis presented by Tanaka et al., that stipulates that Met136Val mutant $Na_V1.6$ channels exhibit more frequent and numerous action potentials than wild-type $Na_V1.6$ channels.

In Figure 4A, an experiment was done to observe the effects of current magnitudes 1X, 2X, and 3X the current threshold on voltage in TRG neurons expressing wild-type and Met136Val mutant channels. It is shown in Figure 4A that as the factor of current threshold increases (1X, 2X, 3X), more action potentials are fired in the same amount of time with the Met136Val mutant firing more at each factor of current threshold. For instance, there is a higher number of action potentials at 3X the current threshold than at 2X in both wild-type and Met136Val mutant neurons, but when looking at the 3X trial specifically, the Met136Val mutant fires many more action potentials than the wild-type channel. In Figure 4B, it is observed that for the same amount of injected current (between 100 pA and 475 pA), there was a significant

increase in spiking during the 500ms current injection for the Met136Val mutant than the wild-type channel. This significant increase in spiking number indicates a major increase in excitability in Met136Val mutants, supporting the hypothesis of hyperexcitability. In Figure 4C, the number of maximal spikes in wild-type and Met136Val mutant channels is counted. In low-firing neurons (below their respective lines), both the wild-type and Met136Val mutant (8 cells each) fired 1-6 action potentials each. The amount of firing is about the same in low-firing neurons, regardless of whether they are wild-type or Met136Val mutant. However, in high-firing neurons (above their respective lines), the 10 wild-type cells fired between 8-21 action potentials each, with only 2 cells firing above 15 action potentials each, while the 14 mutant cells fired over 15 action potentials each. It is observed that there are more Met136Val mutant cells than wild-type cells firing above 15 action potentials (at fractions of 14/22 and 2/18, respectively). Therefore, it is evident that Met136Val mutant TRG high-firing neurons fire more action potentials than wild-type TRG high-firing neurons. As aforementioned, the higher number of action potentials is associated with NVC. All of the data presented in Figures 4A, 4B, and 4C corroborate the hypothesis, as all Met136Val mutants are more excitable than wild-type channels in the sense that they consistently fire more action potentials.

There is a definite shortcoming related to the breadth of the impact that this experiment will have on the treatment and diagnosis of TN, in part due to the fact that TN can be caused by multiple factors (genetic and epigenetic) and does not necessarily come with NVC. The current treatment method for TN is the use of voltage-gated sodium channel blockers such as carbamazepine and oxcarbazepine. Since these blockers affect all voltage-gated sodium channels and they succeed in reducing pain in TN patients, the discovery that specifically

channel $Na_V1.6$ is involved in TN does not affect the current treatment plan. In terms of presentation of the data, more figures and photos would have been useful in clarifying the setup of the molecular genetics aspects of the experiment. Another caveat is that all experiments were performed in mouse and rat neurons, so although they are structurally similar to human neurons, it is impossible to verify with certainty that human and animal model neurons respond in the same ways to these experiments without testing on human neurons. Therefore, a human trial would be necessary in the later stages of research in the impacts of $Na_V1.6$ on TN in order to validate the results presented by Tanaka et al., before moving on to concrete applications of the finding, such as testing a $Na_V1.6$ blocking drug. An example of a potential future treatment for TN, if it is established that TN is consistently and directly related to a gain-of-function mutation in $Na_V1.6$, would be to use 4,9-anhydroTTX, proven to selectively block the activity of $Na_V1.6$ channels with high efficacy (Teramoto, Yotsu-Yamashita, 2015). In future experiments, it would be interesting to see whole-exome sequencing of other patients with diagnosed TN, in order to observe if they also had mutations in their SCN8A exons (which encode $Na_V1.6$). These experiments would determine whether TN was specifically related to $Na_V1.6$, or if other sodium channels might be mutated in different patients diagnosed with TN.

Works Cited

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