Waxman Lab EM Research Update – January 2011

Our multidisciplinary efforts to unravel the molecular drivers of pain are continuing to yield promising new discoveries that bring us closer to effective treatments for EM.

Recent highlights of our research include:

We are making remarkable strides in understanding the molecular genetics of the EM gene: Within just a few years after our initial demonstration of the role of sodium channel Nav1.7 in EM, several dozen different mutations in Nav1.7 have surfaced and been shown to cause three distinct disease syndromes. One set of mutations produces Nav1.7 channels that are "quick to turn-on", in a manner that greatly increases their ability to respond to slightest perturbations. Such mutant Nav1.7 channels cause the characteristic burning pain and redness of EM. Another set of mutations impair the "turn-off" mechanism of Nav1.7 channels such that they are continuously "in high gear", producing an increased and persistent response that causes an extreme pain disorder called PEPD, a perirectal, periocular, and perimandibular pain, triggered by perineal stimulation. A third set of "loss-of-function" mutations cause congenital insensitivity to pain, in which, affected individuals lack functioning Nav1.7 channels and do not feel pain. This last group of mutations is important because it teaches us that, by controlling the ability of Nav1.7, it should be possible to alleviate pain due to many causes, including sporadic and secondary EM. These multiple disease manifestations, all due to mutations in Nav1.7, strongly implicate Nav1.7 as a unique target for the pharmacotherapy of pain in humans. We are working with pharmaceutical companies toward this goal.

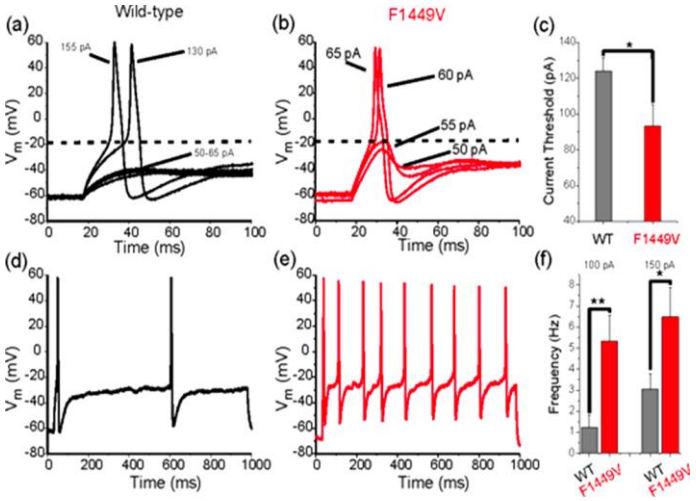

Figure 1: Nav1.7 gain-of-function EM mutation F1449V makes pain signaling (DRG) neurons hyperexcitable. *(a,b,c) Current threshold (c) for generation of single action potentials is reduced in DRG neurons expressing F1449V gain of-function Nav1.7 (b) compared with WT channels (a). (d,e,f) Mutant channels also produce increased firing frequency in response to small stimuli (e) compared with wild-type (d). * p<0.05; ** p<0.01.*

Estacion, M., Dib-Hajj, S.D., Benke, P.J., te Morsche, R.H.M., Eastman, E.M., Macala, L.J., Drenth, J.P.H., and Waxman, S.G. NaV1.7 gain-of-function mutations as a continuum: A1632E displays physiological changes associated with erythromelalgia and paroxysmal extreme pain disorder mutations and produces symptoms of both disorders, J. Neurosci., 28(43): 11079-11088, 2008.

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Estacion, M., Choi, J.S., Eastman, E. Lin, Z., Li, Y., Tyrrell, L., Yang, Y., Dib-Hajj, S., Waxman, S.G. Can robots patch-clamp as well as humans? Characterization of a novel sodium channel mutation. J. Physiol., in press, 2010.

Choi, J.S., Cheng, X., Foster, E., Leffler, A., Tyrrell, L., Te Morsche, R.H., Eastman, E.M., Jansen, H.J., Huehne, K., Nau, C., Dib-Hajj, S.D., Drenth, J.P., Waxman, S.G. Alternative splicing may contribute to time-dependent manifestation of inherited erythromelalgia. Brain 133:1823-1835, 2010.

Cheng, X., Dib-Hajj, S.D., Tyrrell, L., Wright, D.A., Fischer, T.Z., and Waxman, S.G. Mutations at opposite ends of the DIII/S4-S5 linker of sodium channel Nav1.7 produce distinct pain disorders. Molec. Pain, 2010 Apr 29;6:24..

Ahn, H-S, Dib-Hajj, S., Cox, J.J., Tyrrell, L., Elmslie, F.V., Clarke, A.A., Drenth, J.P.H., Woods, C.G., Waxman, S.G. A new Nav1.7 sodium channel mutation I234T in a child with severe pain. Eur. J. Pain, Eur J Pain. 2010 Oct;14(9):944-50. Epub 2010 Apr 10.

Our research on the pharmacogenomics (the study of how genes affect a person's response to drugs) of EM pain is paving the way for personalized medicine: It is well known that EM patients respond to pain medications in different ways. Some may respond well to lidocaine, others to carbamazepine, still others to neither. To understand why, we analyzed mutant Nav1.7 channels in patients with inherited EM, and found single amino acid substitutions that can either reduce, or enhance, their response to drugs such as lidocaine, mexiletine and carbamazepine (Sheets et al., 2007; Choi et al., 2009; Fischer et al., 2009).

Our findings indicate that genetic variations, or polymorphisms within the EM gene, while not producing the disease per se, may alter responsiveness of mutant Nav1.7 channels to pharmacotherapeutic agents. Pharmacogenomic characterization of polymorphisms may permit identification of patients who are more likely to respond favorably to particular treatment than others while paving the way for genetically-tailored, personalized medicine.

Choi, J-S., Zhang, L., Dib-Hajj, S.D., Han, C.Y., Tyrrell, L, Lin, Z., Wang, X., Yang, Y., and Waxman, S.G., Mexiletine-responsive erythromelalgia due to a new Nav1.7 mutation showing use-dependent current fall-off. Experimental Neurology, 16(2):383-9, 2009.
Fischer, T.Z., Gilmore, E.S., Estacion, M, Eastman, E, Taylor, S, Melanson, M, Dib-Hajj, S, Waxman, S.G. A novel Nav1.7 mutation producing carbamazepine-responsive erythromelalgia. Ann Neurol. 65:733-741, 2009.

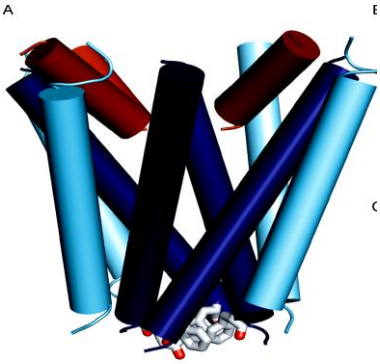

Figure 2: Molecular Modeling of the EM gene. *Our modeling has identified four residues that contribute to the pore aperture gate which stabilizes the closed-state of Nav1.7. Since slow closed-state inactivation is critical for the function of Nav1.7 within pain-signaling neurons, this result will contribute to the understanding of the role of Nav1.7 in pain.*

We have found that polymorphisms or variants of the EM gene, while not causing the disease per se, can affect a person's susceptibility to pain: In addition to affecting drug responsiveness, we have found that polymorphisms in Nav1.7 can also alter excitability of pain-signaling sensory neurons and modulate pain sensitivity. In this investigation, we identified a single-nucleotide polymorphism within the Nav1.7 gene, and showed that the presence of such a polymorphism increases, by 2-fold, their firing frequency of Nav1.7 in response to stimulation. We also found that this "excitability increasing" polymorphism is present in 30% of the normal population, increasing their susceptibility to pain.

Estacion, M., Harty, T.P., Choi, J.S., Tyrrell, L., Dib-Hajj, S.D., Waxman, S.G. A sodium channel gene SCN9A polymorphism that increases nociceptor excitability. Ann Neurol. 66(6): 862-6, 2009.

We are continuing to investigate the basis for age-dependent manifestation of inherited EM to understand what keeps people from developing EM until a certain age. Our prediction is that there are "gating mechanisms" that can suppress pain in EM under some circumstances, and we are actively investigating this.

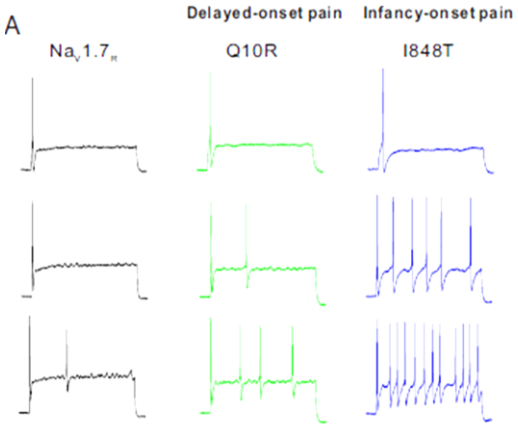

Figure 3: Q10R (clinical onset in 2nd decade) and I848T (infancy onset) EM mutations increase firing frequency in pain-signaling neurons, but to different degrees. *(A) Response of cells expressing WT, Q10R and I848T channels, to 1s depolarizing current steps at 1X, 2X and 3X (top, middle and bottom, respectively) the action potential current threshold*

Han, C., Dib-Hajj, S.D., Lin, Z., Li, Y., Eastman, E.M., Tyrrell, L, Cao, X., Yang, Y., Waxman, S.G. Early-and late-onset inherited erythromelalgia: genotype-phenotype correlation. Brain, 132:1171-1723.2009. PMID 19369487. Choi, J.S., Cheng, X., Foster, E., Leffler, A., Tyrrell, L., Te Morsche, R.H., Eastman, E.M., Jansen, H.J., Huehne, K., Nau, C., Dib-Hajj, S.D., Drenth, J.P., Waxman, S.G. Alternative splicing may contribute to time-dependent manifestation of inherited erythromelalgia. Brain 133:1823-1835, 2010. PMID: 20478850

- We are committed to shortening the distance between discovery research and delivery of treatments to EM patients, and are working feverishly across international and institutional boundaries in a multinational battle against EM. The two largest centers for EM research in China (led by Dr. Yong Yang) and Europe (led by Dr. Joost Drenth) both send us DNA of EM patients for analysis, not only giving us

access to precious information gleaned from rare but highly informative patients but also allowing us to work back and forth between clinical and basic research.

- We are extremely grateful for TEA's support which is both invaluable and essential in this battle against EM. The research progress noted above is undoubtedly the result of a synergistic effort and would not be possible with individual capabilities.