Journal Club

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An iPSC Model Reveals Mechanisms of Interindividual Differences in Pain

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Review of Mis et al.

How humans experience pain varies greatly between individuals; something that is innocuous to one person can be very painful for another. This variability exists among healthy individuals but looms largest for the millions of people affected by chronic pain. The development of chronic pain appears to have a substantial heritable component (Hocking et al., 2012; Nielsen et al., 2012; Zorina-Lichtenwalter et al., 2016), and genetic causes are thought to underlie at least some of the variability in how individuals experience pain. But because pain is a complex and multifaceted process, linking human genetics to pain phenotypes has proven difficult. Nevertheless, major progress toward this challenge has been achieved through the study of rare familial pain disorders.

Many inherited pain disorders involve mutations of the voltage-gated sodium channel $Na_v1.7$ (Dib-Hajj et al., 2013). Because expression of $Na_v1.7$ is essentially restricted to one locus in pain pathways (i.e., peripheral nociceptors), a relatively straightforward theory of these diseases has emerged. Mutations that alter the function of $Na_v1.7$ change the electrophysiological properties of nociceptors, altering their output in a way that produces dysfunctional pain signaling (Drenth and Waxman, 2007). For example, in inherited erythromelalgia (IEM), a disorder characterized by attacks of burning pain in the extremities, gain-of-function mutations in $Na_v1.7$ facilitate its activation and thus increase nociceptor excitability (Harty et al., 2006; Drenth and Waxman, 2007; McDonnell et al., 2016). Consequently, the nociceptors become active spontaneously or in response to normally innocuous triggers, leading to pain attacks. Mysteriously, however, individuals harboring the IEM-linked $Na_v1.7$ mutations can experience substantially variable pain symptoms (Geha et al., 2016; McDonnell et al., 2016).

A recent study by Mis et al. (2019) set out to study how interindividual differences in the manifestation of IEM arise. They performed a comparative study on three members of a family affected by IEM: a mother, a father, and a son. The mother and the son both harbor a $Na_v1.7$ mutation ($Na_v1.7$-S241T) and experience IEM symptoms, but the pain profile of the son is considerably more severe (Geha et al., 2016; McDonnell et al., 2016; Mis et al., 2019). The father lacks the mutation and is unaffected by IEM.

The authors hypothesized that differences in peripheral nociceptors might play a role in the different pain profiles. To address this, they used a "disease-in-a-dish" model in which they generated induced pluripotent stem cells (iPSCs) from patient blood samples and then induced the cells to differentiate into sensory neurons (SNs) that resemble peripheral nociceptors. The use of iPSC-SNs made it possible to perform a detailed analysis of the electrophysiological phenotypes of neurons from each of these patients.

Recording with multielectrode arrays and whole-cell patch clamp, the authors found dramatic differences in the excitability of iPSC-SNs from the three family members, which corresponded to the severity of their pain phenotypes. The iPSC-SNs from the mutation-carrying people were much more excitable and exhibited higher levels of spontaneous activity compared with the father. Moreover, the iPSC-SNs from the (severe pain phenotype) son were substantially more excitable than those from the (moderate pain phenotype) mother. These findings lend support to the validity of this system as a model of subject-specific nociceptor dysfunction, albeit for a small sample of patients.

The authors next sought to determine the mechanisms causing the son's iPSC-SNs to be more excitable than those of his mother. They found that the resting membrane potential (RMP) of the iPSC-SNs derived from the son was several millivolts more depolarized than iPSC-SNs from either parent. Intriguingly, simply

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injecting a hyperpolarizing current into the son's iPSC-SNs (or injecting a depolarizing current into the mother's) largely abolished the differences in their excitability. This suggested that a second factor, separate from $Na_v1.7$, might be present and driving differences in RMP between the mother and son.

The authors searched for such a factor using whole exome sequencing. This revealed a novel variant in *KCNQ2*, the gene encoding the voltage-gated potassium channel $K_v7.2$, in the mother but not the son. $K_v7.2$ contributes to the noninactivating I_M current (Passmore et al., 2003), which affects the RMP, so the authors next tested how I_M might be affected by this mutation. Pharmacologically isolating I_M, they determined that the $K_V7.2$-T730A mutation causes a hyperpolarizing shift in the activation threshold of the I_M current. From this shift, one would predict that I_M conductance would be comparatively larger over a wide range of voltages below the action potential threshold, hyperpolarizing the RMP. The authors confirmed this hypothesis using dynamic clamp to approximately cancel out the $K_V7.2$-T730A conductance while substituting the WT $K_v7.2$ conductance in the mother's iPSC-SNs. This depolarized the iPSC-SNs' RMP to a level similar to that seen in the son's iPSC-SNs. Thus, in the context of an existing $Na_v1.7$ channelopathy, $K_V7.2$-T730A acts as a protective mutation that attenuates nociceptor hyperexcitability and likely contributes to the reduced severity of the mother's pain profile.

This study demonstrates an exciting approach that bridges human genetics, physiology, and patient outcomes. Many diseases are dauntingly complex, leading investigators to search for intermediate phenotypes or "endophenotypes" (Gottesman and Gould, 2003) that are associated with pathology, but more readily measured and connected with underlying genetic causes. The disease-in-a-dish model of nociceptor excitability used by Mis et al. (2019) might be thought of as an "ectophenotype," a disease-related signature that can be measured in a biological system outside of the patient. Neurons are rich dynamical systems; but in controlled experimental settings, their electrophysiological behavior is highly reliable and the underlying mechanisms often can be understood with careful analysis. Thus, measurements of neuronal intrinsic properties can be highly informative and are potentially powerful ectophenotypes to assay in patient-specific model systems. Mis et al. (2019) have demonstrated this by establishing a mechanism for the interaction between two rare mutations in the context of IEM.

Similar iPSC-based approaches have been used to study other channelopathies, such as congenital insensitivity to pain (McDermott et al., 2019) and epilepsy (Sun et al., 2016; Thodeson et al., 2018). In addition to furthering our understanding of basic mechanisms of disease, examining interindividual differences in populations affected by these conditions could yield insight into why drugs prescribed as treatments are more effective for some individuals than others and could perhaps even one day contribute to personalized therapies.

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