phenotypes. These mutations cluster around the gate of the ion channel and likely increase current. By contrast, we also observed a class of mutations that act as dominant-negatives (antimorphs) in the nematode. Animals with these mutations display a stereotyped "fainting" behavior. Antimorphic channels likely cause the aggregation and degradation of wild-type NALCN, similar to mutations described in related sodium and calcium channels.[22,23] Thus, 3 mechanisms give rise to NALCN channelopathies: (1) IHPRF—recessive loss-of-function, (2) CLIFAHDD—dominant gain-of-function, and (3) CLIFAHDD—dominant antimorphic.

Unfortunately, the human phenotypes that result from the different genetic mechanisms of NALCN pathology are not so easily placed into these categories. IHPRF and CLIFAHDD "syndromes" are characterized by dysmorphic features and neurodevelopmental disease with a significant number of shared features. Both IHPRF and CLIFAHDD patients display regional hypotonia and intellectual disability, and members of each group experience seizures. The most consistent feature attributed exclusively to CLIFAHDD syndrome is distal arthrogryposis (reported in 18 of 19 patients described to date) (figure 1).[9–11] Distal contractures were not noted in the 11 patients described with IHPRF.[6–8] Our results complicate this picture further, since it appears that gain- or loss-of-function mutations in *NALCN* can lead to a CLIFAHDD diagnosis. These results are consistent with the overlap between CLIFAHDD and IHPRF but at odds with the distinctive feature such as arthrogryposis. Given that NALCN is expressed in excitatory and inhibitory neurons, both sides of a balanced circuit will be affected by functional changes to the channel. Therefore, the motor output of a given circuit is difficult to predict and likely reflects homeostatic limits to the system. Therefore, the degree of phenotypic overlap between these syndromes may not be surprising.

The phenotypes observed in CLIFAHDD and IHPRF do suggest that the NALCN ion channel functions very broadly in the nervous system. Sustained muscular hyperactivity and failure of relaxation of limb and cranially innervated muscles, in conjunction with the observed rhythmic cycling activity of the limbs, suggest that this ion channel functions in both upper and lower motor neurons and associated circuitry in the peripheral nervous system as well as the CNS. Hyperexcitability of the motor unit and the resulting overactivity of muscles innervating both limbs and cranial structures, if present during fetal development, help to explain the characteristic dysmorphology and distally predominant congenital contractures observed in these patients.

Redefining the mechanisms of CLIFAHDD syndrome changes the approach for treating these patients. Gain-of-function variants could be targeted with ion channel blockers to decrease cellular excitability. Further functional studies are needed to identify specific blockers of NALCN. However, current medications in use for epilepsy or other indications may prove to be valuable candidates. Alternatively, NALCN variants identified with a loss-of-function mechanism may benefit from a global increase in cellular excitability. Unfortunately, both avenues will require extensive investigation and clinical trials. What is critical is that care providers practice caution before delivering such drugs because different patients with CLIFAHDD may respond in dramatically different ways. In particular, testing specific variants in *C elegans* may lead to more accurate diagnoses and drug treatments.

Comment: Genotype–phenotype correlation with CRISPR-Cas9—Bedside to bench

Technological improvements and decreasing costs have led to increased use of next-generation sequencing as an a priori approach to clinical diagnosis. This approach lends itself to important discoveries of novel genotypic etiologies and phenotypic associations.

In the current report, the authors present a case of congenital arthrogryposis in an infant with a de novo missense mutation in the *NALCN* gene identified with whole-exome sequencing.[1] Two groups originally reported *NALCN* mutations in 2013 in association with congenital contractures of the limbs and face with hypotonia and developmental delay (CLIFAHDD) syndrome.[2,3] In contrast to earlier reports, Bend et al. describe in their patient the clinical electrophysiologic features of peripheral motor system hyperexcitability, thus expanding the phenotypic spectrum of *NALCN*-related disorders.

These findings are further investigated by probing the functional consequences of the orthologous missense *NALCN* mutation from their patient, as well as other previously reported *NALCN* mutations, using the CRISPR-Cas9 system in the model organism *Caenorhabditis elegans*. Consistent with their patient's clinical features of peripheral motor system overactivity, the authors nicely demonstrate in *C elegans* a gain of function as a consequence of the patient's mutation. Furthermore, other mutations previously reported in association with CLIFAHDD also had either loss- or gain-of-function consequences.

The authors' approach is an excellent example of how to use the CRISPR-Cas9 in a model system to investigate the functional consequences of missense mutations. The authors' use of motor behavior of *C elegans* as a straightforward readout and the conserved nature of the *NALCN* gene makes the studies more easily interpreted. However, this paradigm may be less suited to the study of other disorders with more complex phenotypic–genotypic relationships, or in the study of less well conserved genes.

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