Zebrafish as a Model System for the Study of Severe \( \text{Ca}_V2.1 (\alpha_{1A}) \) Channelopathies

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The \( \text{P/Q-type \text{Ca}_V2.1} \) channel regulates neurotransmitter release at neuromuscular junctions (NMJ) and many central synapses. \( \text{CACNA1A} \) encodes the pore-containing \( \alpha_{1A} \) subunit of \( \text{Ca}_V2.1 \) channels. In humans, \textit{de novo \text{CACNA1A}} mutations result in a wide spectrum of neurological, neuromuscular, and movement disorders, such as familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2), as well as a more recently discovered class of more severe disorders, which are characterized by ataxia, hypotonia, cerebellar atrophy, and cognitive/developmental delay. Heterologous expression of \( \text{Ca}_V2.1 \) channels has allowed for an understanding of the consequences of \( \text{CACNA1A} \) missense mutations on channel function. In contrast, a mechanistic understanding of how specific \( \text{CACNA1A} \) mutations lead \textit{in vivo} to the resultant phenotypes is lacking. In this review, we present the zebrafish as a model to both study \textit{in vivo} mechanisms of \( \text{CACNA1A} \) mutations that result in synaptic and behavioral defects and to screen for effective drug therapies to combat these and other \( \text{Ca}_V2.1 \) channelopathies.

Keywords: \( \text{Ca}_V2.1 \), \( \alpha_{1A} \), \text{P/Q-type}, channelopathy, familial hemiplegic migraine type 1, episodic ataxia type 2, vertebrate models, zebrafish

INTRODUCTION

\( \text{P/Q-type Ca}_V2.1 \) channels are the predominant voltage-gated \( \text{Ca}^{2+} \) channel isoform present at the neuromuscular junction (NMJ) and most central synapses. Since \( \text{Ca}^{2+} \) flux via these channels is critical for neurotransmitter release (Llinás et al., 1981; Turner et al., 1992; Uchitel et al., 1992; Dunlap et al., 1994, 1995; Ludwig et al., 1997), mutations in the \( \text{Ca}_V2.1 \alpha_{1A} \) subunit would be expected to impact synaptic efficacy. However, as discussed in sections “\( \text{Ca}_V2.1 \) Channel Composition” to “The Expanding Spectrum OF \( \text{Ca}_V2.1-\alpha_{1A} \) Channelopathies” the direct consequences of mutations on channel function and the resultant neurologic phenotypes vary significantly. For example, two well-studied channelopathies—episodic ataxia type 2 (EA2) and familial hemiplegic migraine type 1 (FHM1)—arise from point mutations in the \( \text{CACNA1A} \) gene that encodes the \( \alpha_{1A} \) subunit (Jen et al., 2007; Pietrobon, 2007, 2010).
The mutations that lead to EA2 tend to be loss-of-function mutations, while gain-of-function mutations usually underlie FHM1 (Jen et al., 2001; Tottene et al., 2002; Kaja et al., 2005, 2010; Mantuano et al., 2010; Rajakulendran et al., 2010b; Di Guilm et al., 2014; Rose et al., 2014; Brusch et al., 2018). However, some ataxic cases have paradoxically been linked to gain-of-channel function mutations (e.g., van den Maagdenberg et al., 2010; Knierim et al., 2011; Gao et al., 2012; Bahamonde et al., 2015; Jiang et al., 2019). These latter examples underscore the diversity of channel dysfunction in this expanding spectrum of ataxic disorders and highlight the need for a model system to rapidly and effectively identify pathological phenotypes.

In this article, we review the: (1) basic information about the Cav2.1 channel heteromultimer; (2) two relatively well-characterized diseases caused by mutation of the Cav2.1 α1A subunit—EA2 and FHM1; (3) the emerging full spectrum of Cav2.1 α1A channelopathies; and (4) the potential that the zebrafish model holds for understanding disease mechanisms and discovering potential therapeutics. Sections “Introduction” to “Familial Hemiplegic Migraine Type 1” are intended to provide sufficient background for the more profound discussion of the more severe neurodevelopmental disorders, which are caused by point mutations in CACNA1A in section “The Expanding Spectrum of Cav2.1-α1A Channelopathies.” It is important to note that the pathology of this unnamed class of disorders resembles that of spinocerebellar ataxia type 6 (SCA), which is caused by the addition of excess CAG polynucleotide repeats to the CACNA1A transcript (Jodice et al., 1997).

**Cav2.1 CHANNEL COMPOSITION**

High voltage-activated Ca\(^{2+}\) channels, such as the Cav2.1 heteromultimer, are composed minimally of a principal α1 subunit (α1A) and auxiliary β and α2δ subunits (Volsen et al., 1997; Catterall, 2010; Dolphin, 2016). For Cav2.1, an interaction with a γ2 subunit (a.k.a., stargazin) was also reported (Letts et al., 1998; Kang and Campbell, 2003). Like the other nine members of the Cav family, α1A subunits have four transmembrane repeats (I–IV), each with six membrane-spanning α-helices (S1–S6; Mori et al., 1991; please see Figure 1). Of these, the S4 α-helices are thought to be the primary voltage-sensing elements of the channel, a function which is conferred by five to six positively charged amino acids lining a face of the α-helix (Aggarwal and MacKinnon, 1996). The S1–S3 helices form an aqueous conduit that enables passage of the S4 α-helix through the membrane field by facilitating interactions with residues of the “charge transfer center” (formed by conserved negative, polar and hydrophobic residues on the S2 segment and an invariant aspartate residue on the S3 helix; Tao et al., 2010); the S5 and S6 helices line the conventional channel conduction pore (Neely and Hidalgo, 2014; Hering et al., 2018). The relatively long extracellular segment linking the S5 and S6 helices (a.k.a., the P-loop) contains a highly conserved glutamate residue in all four repeats. These four glutamates form the selectivity filter (Yang et al., 1993).

**EPISODIC ATAXIA TYPE 2**

EA2 is a rare neurological disease characterized by paroxysmal attacks of ataxia, nystagmus, and vertigo. The majority of CACNA1A mutations that lead to EA2 result in Cav2.1 loss of function by premature termination of the open reading frame, resulting in rapid degradation of truncated protein products (Jen et al., 2001; Pietrobon, 2010; Sintas et al., 2017). Indeed, over 40 pathogenic missense mutations were identified (Pietrobon, 2010; Sintas et al., 2017; see Figure 1). Most of these amino acid substitutions reside in the P-loop or the S5 and S6 helices, themselves, suggesting that impaired ability to form a fully functional channel pore is the likely pathophysiological mechanism of the resultant phenotype for the majority of EA2 missense cases (Jen et al., 2007; Sintas et al., 2017). In some cases, a complete loss of function was observed with missense mutants, likely attributable to ER-associated degradation of the mutant channel and subsequent lack of trafficking to the surface membrane (Page et al., 2004). In addition, some EA2 mutants (e.g., E1761K, F1406C) seem to exert a dominant-negative effect since coexpression of mutant channels with wild-type channels in Xenopus oocytes diminished the amplitude of Ca\(^{2+}\) current elicited by depolarization (Jeng et al., 2006, 2008; Mezghrani et al., 2008). In these latter cases, it was postulated that misfolded mutant channels bound wild-type channels and subsequently induced degradation (Page et al., 2010; Rajakulendran et al., 2012; Dahimene et al., 2016) or competed successfully with the wild-type channel for a limited number of “slots” reserved for Cav2.1 channels at the plasma membrane (Cao et al., 2004; Cao and Tsien, 2010; but see below). In addition, some mutations (e.g., H1736L, A1293D/delY1294, G293R) do not completely abolish channel activity but rather shift the voltage-dependence of Cav2.1 activation to somewhat more positive potentials, thereby decreasing channel open probability (P\(_o\); Wappl et al., 2002; Spacey et al., 2004; Pietrobon, 2010).

In a minority of cases, EA2 is precipitated by gain-of-channel function mutations, which suggests that a critical bandwith of Ca\(^{2+}\) flux is required to avoid pathogenicity (e.g., Mantuano et al., 2010; Knerim et al., 2011; Gao et al., 2012; Carreño et al., 2013; Bahamonde et al., 2015). For many yet-to-be characterized Cav2.1 EA2 mutations, whether the mutation produces gain- or loss-of-channel function remains to be seen. Still, these findings underscore the need to resist generalization regarding pathological mechanisms without rigorous investigation of each mutation.

**FAMILIAL HEMIPLEGIC MIGRAINE TYPE 1**

FHM1 is an inherited migraine condition that results in weakness of half the body for prolonged periods of time. Patients afflicted with FHM1 often display cerebellar degeneration (Elliot et al., 1996). As noted above, FHM1 is most often linked to gain-of-function point mutations in CACNA1A (Tottene et al., 2002; Pietrobon, 2007; see Figure 2). These substitutions occur at a variety of loci within the channel but most commonly in residues...
FIGURE 1 | Schematic representation of human Ca$_{V}$2.1 mutations causing episodic ataxia type 2 (EA2). Please note that residue numbering varies between studies due to the existence of multiple CACNA1A splice variants; residue numbers indicated reflect those stated in the original report. Citations to the indicated mutations are listed as follows: E147K—Imbrici et al., 2004; G162V—Maksemous et al. (2016); R192W—Soden et al. (2014); R198Q—Indelicato et al. (2019); Y248C—Zafeiriou et al. (2009); Y248N—Choi et al. (2017); H253Y—van den Maagdenberg et al. (2002); C256R—Mantuano et al. (2004); R279C—Maksemous et al. (2016); C287Y—Jen et al. (2004); G293R—Yue et al. (1997); G297R—Tantsis et al. (2016); D302N—Maksemous et al. (2016); R387G—Maksemous et al. (2016); E388K—Nikaido et al. (2011); L389F—Mantuano et al. (2010); G411W—Maksemous et al. (2016); C256R—Mantuano et al. (2004); R2090Q—Melzer et al. (2010); R2136C—Mantuano et al. (2004); P2222L—Sintas et al. (2017). The Ca$_{V}$2.1 schematic was modified from Tyagi et al. (2019) with permission of the authors.

thought to line the pore, the S3–S4 or S5–S6 linkers, or the S4 voltage sensor. Even though the locations of the mutations within the channel are variable, analysis in heterologous systems revealed a hyperpolarizing shift in channel activation for most studied mutants (Hans et al., 1999; Tottene et al., 2002, 2005; Adams et al., 2009; Serra et al., 2009). Since these channels open at more hyperpolarizing potentials, channel $P_o$ is enhanced, and an FHM1 mutant Ca$_{V}$2.1 channel can carry greater Ca$^{2+}$ influx than its wild-type counterpart at physiologically relevant membrane potentials. This process may be further facilitated by a reduction in the direct $G_{\beta\gamma}$-mediated inhibition of presynaptic FHM1 mutant Ca$_{V}$2.1 channels (Melliti et al., 2003; Weiss et al., 2008; Serra et al., 2009; Garza-López et al., 2012, 2013). Mouse knock-in models carrying FHM1-causing Ca$_{V}$2.1 mutations display the migraine aura, cortical spreading depression characteristic of human FHM1 (van den Maagdenberg et al., 2004, 2010). While these gain-of-function biophysical effects of FHM1 mutations are fairly consistent, it is important to state that FHM1 pathology is inarguably a reflection of the balance of the relative manifestation of the mutations between excitatory and inhibitory circuits (Vecchia et al., 2015).

THE EXPANDING SPECTRUM OF Ca$_{V}$2.1-$\alpha_{1A}$ CHANNELOPATHIES

EA2 and FHM1 have long been known to be caused primarily by point mutations in Ca$_{V}$2.1 in addition to a few variants that carry deletions or insertions (Jen et al., 2001; Pietrobon, 2007, 2010). However, the biophysical effects of these mutations on channel function are often subtle, and the manifestations of ataxia are paroxysmal.
FIGURE 2 | Schematic representation of human Ca\textsubscript{V}2.1 mutations causing familial hemiplegic migraine type 1 (FHM1). Please note that residue numbering varies between studies due to the existence of multiple CACNA1A splice variants; residue numbers indicated reflect those stated in the original report. Citations to the indicated mutations are listed as follows: R192Q—Ophoff et al. (1996); R195K—Ducros et al. (2001); S218L—Kors et al. (2001); P225H—Stuart et al. (2012); G230V—Yang et al. (2014); F363S—Riant et al. (2010); V581M—Cuenca-León et al. (2008); V714A—Ophoff et al. (1996); D715E—Ducros et al. (2001); E1015K—Grieco et al. (2018); Y1245C—Cuenca-León et al. (2008); K1336E—Ducros et al. (2001); R1347Q—Alonso et al. (2004); C1535S—Dichgans et al. (2005); F1609L—Pelzer et al. (2018); R1668W—Ducros et al. (2001); K1670R—Riant et al. (2010); L1682P—Weiss et al. (2007); W1684R—Ducros et al. (2001); I1710T—Kors et al. (2004); D1725N—Riant et al. (2010); I1811L—Ophoff et al. (1996); A2006T—Wilson (2014); R2157G—Grieco et al. (2018). The Ca\textsubscript{V}2.1 schematic was modified from Tyagi et al. (2019) with permission of the authors.
I–II linker, A454T, was demonstrated to curb modulation of Cav2.1 by SNARE proteins via a mechanism involving the β subunit (Cricchi et al., 2007; Serra et al., 2010, 2018).

Blumkin et al. (2010) reported a R1350Q substitution in a 7-year-old male patient that also presented with cerebellar ataxia, developmental delay, and nonspecific dyskinesia. Although the outward presentation was similar to the patient carrying the A405T substitution, the R1350Q swap inserted a neutral glutamate in place of a basic arginine in the S4 voltage-sensing α-helix of Repeat III (Figure 1). An arginine to glutamine substitution at this position was also reported with a patient exhibiting tremor that was alleviated by a Ca\(^{2+}\) channel blocker (R1345Q in Jiang et al., 2016). Based on the observation that the equivalent substitution in the \(\alpha\) subunit of Repeat III (P1353L) found in a child of Weyhrauch et al. (2016), who also described a mutation in the S4 voltage sensor of Repeat III (\(\alpha\)CAV1A allele seems normal (Jun et al., 1999). However, the ability of Cav2.1P1353L to out-compete endogenously wild-type channels was not investigated in a neuronal context.

Travaglini et al. (2017) reported a pair of mutations, I1342T and V1396M, in two patients with similar clinical phenotypes involving congenital ataxia, hypotonia, and intellectual disability. The I1342T mutation resides in the extracellular loop between the S3 and S4 helix of \(\alpha\)I, a region of the channel that is also predicted to interact with the \(\alpha\)δ subunit on the basis of Cav2.1 cryo-EM structure (Wu et al., 2016). The idea that V1396M facilitates channel expression through an \(\alpha\)δ-mediated mechanism (see Dolphin, 2016, for a review) is particularly intriguing since the current density for the mouse equivalent of Cav2.1 V1396M expressed in HEK293 cells was shown to be nearly double that of wild-type Cav2.1 (jiang et al., 2019). Though less striking, the introduction of methionine also causes a hyperpolarization in the voltage dependence of activation suggesting the disruption of an inter-helical interaction that restricts voltage-sensor translocation. Three other Cav2.1 mutants, which were linked to Lennox–Gastaut epileptic encephalopathy were examined in the same study and were found to have polar effects (jiang et al., 2019). The A715T mutation at the base of RII56 displayed a ~10-mV hyperpolarizing shift in activation, smaller but reminiscent of the ~20-mV hyperpolarizing shift observed in Purkinje cells of Cav2.1 S218L EA2 model mice (Gao et al., 2012). On the other hand, G232V and I1357S, at the bases of RII5 and RII4 helices, respectively, reduced channel plasma membrane expression in both HEK293 and in cortical neurons.

Seminal work from Richard Tsien’s laboratory in the early 1990s revealed that four highly conserved glutamate residues within the P-loop are the structural basis of Ca\(^{2+}\) selectivity among all Cav channels (Yang et al., 1993). Two such mutations in \(\alpha\)I are known to occur at the same glutamate in Repeat IV. Mutation of this residue to glycine causes ataxia and cognitive deficits running through three generations of the Slovak family (E1755G in Petrovicova et al., 2017), and as noted above, a reversal of charge via substitution of a lysine for the glutamate causes EA2 (E1761K in Denier et al., 2001). The glutamate to lysine mutation ablates inward Ba\(^{2+}\) flux \(\text{via} \; \text{Ca}^{2+}\) channels in Xenopus oocytes (Jeng et al., 2006). Since coexpression of the Cav2.1 E1761K mutant with the wild-type channel reduced the amplitude of the current in an RNA dose-dependent manner, the authors postulated that the E1761K resulted in a dominant-negative effect. While this mechanism could certainly underlie this particular channelopathy, conversion of any one of the glutamates in the selectivity filter to lysine effectively transforms Cav2 channels into non-specific monovalent ion channels that are subject to block by divalent ions (Yang et al., 1993). In this regard, Jeng et al. (2006) used a concentration of Ba\(^{2+}\) (40 mM) in their experiments showing the ablation of inward current via E1761K channels, which most likely would have blocked the mutant channel. At more physiological divalent ion concentrations (i.e., <2 mM Ca\(^{2+}\)), currents carried by Na\(^{+}\) and K\(^{+}\) might be visible and pathogenic. Indeed, aberrant Na\(^{+}\) and K\(^{+}\) flux \(\text{via} \; \text{Ca}^{2+}\) Repeat III glutamate to lysine mutant channels can prolong action potential duration in cardiac-like iPSCs (Ye et al., 2019), while the equivalent mutation in Cav2.1 is postulated to cause K\(^{+}\) accumulation in the transverse tubules (Beqollari et al., 2018) and to accelerate muscle fatigue in mice (Lee et al., 2015). Thus, the possibility that the E1761K mutation augments neurotransmitter release by prolonging neuronal action potential duration is not unreasonable, nor is the idea that excessive K\(^{+}\) secretion into restricted extracellular compartments may excite neighboring neurons or vascular smooth muscle cells (see Filosa et al., 2006).

Recently, Luo et al. (2017) described an 8-year-old female patient with congenital ataxia, hypotonia, cerebellar atrophy, and global developmental delay. The trio-based exome sequencing of this patient revealed a de novo missense mutation (R1673P) in the gene for Cav2.1. The mutation resulted in an arginine to proline substitution within the Repeat IV S4 voltage-sensing helix of Cav2.1. The R1673P mutation was predicted to be “probably damaging” by PolyPhen-2, a protein structure prediction software. As a means to identify the molecular mechanism by which R1673P precipitates the clinical phenotype, transgenic flies expressing the Drosophila equivalent of wild-type Cav2.1 and Cav2.1 R1673P in a Cav2.1-deficient Drosophila
(i.e., cacophony mutants) background were generated. In these experiments, the mutant CaV2.1 R1673P was able to rescue the photoreceptor response in 3-day-old larvae to a greater extent than the wild-type channel suggesting a gain-of-function effect. At 30 days, the rescue of the electroretinogram had dissipated, but substantial photoreceptor degeneration was observed in the R1673P line but not in wild-type or CaV2.1-deficient flies. It is possible that the early effects of gain-of-function CaV2+ channel activity triggered neurodegeneration secondary to CaV2+ toxicity. In contrast, however, voltage-clamp experiments showed that the R1673P mutation causes a profound loss-of-function for channels expressed heterologously in tsA-201 cells (Tyagi et al., 2019). Specifically, the rat ortholog of R1673P (R1624P) displayed a ∼25-mV depolarizing shift in activation and resultant weak activation at physiologically relevant membrane potentials. Further work is needed to understand how the loss of function at the molecular level leads to neurodegeneration at the systemic level.

**ZEBRAFISH AS A MODEL SYSTEM FOR THE STUDY OF SEVERE CaV2.1 CHANNELOPATHIES**

Heterologous expression systems are the industry standard for the identification of pathogenic channel dysfunction. However, it is often difficult to extrapolate information gleaned using this approach to neurological dysfunction in patients. To bridge this gap, animal models are employed. Mice carrying FHM1 or EA2 mutations were very useful in understanding the pathophysiology underlying these disorders. However, no mouse line yet exists that models the more severe developmental disorders discussed above. The paucity of such models may be due to the uncertain viability or breeding capability of mice with grave developmental defects and the monetary risk associated with this endeavor. By contrast, simpler organisms like Drosophila have rapid propagation, are relatively easy to manipulate genetically, and lack the burden of cost. The obvious shortcoming of Drosophila is that insects are both phylogenetically and physiologically far removed from humans. A notable shortcoming is that Drosophila lack a true CaV2.1 channel (Smith et al., 1996).

Zebrafish—*Danio rerio*—offers a unique complement to the strengths of flies and mice as models for the study of severe CaV2.1 channelopathies. The zebrafish is useful to investigate mechanisms because of the conservation of most fundamental physiology processes (e.g., neurotransmitter release) with mammals with a reduced risk of embryonic lethality. Similar to many zebrafish genes, the gene encoding the CaV2.1 α-subunit is duplicated, yielding cacna1aa and cacna1ab. Two zebrafish loss-of-function cacna1ab mutants, *tb204a* (Wen et al., 2013) and *fakir* (Low et al., 2012), were studied previously. For both mutations, the loss-of-channel function was sizable, but incomplete. The *tb204a* mutation results in a tyrosine-to-asparagine substitution (Y1662N) within the carboxyl terminus of CaV2.1a and a depolarizing shift in channel activation, similar to what was found for the rat cognate of CaV2.1 R1673P (Tyagi et al., 2019). Homozygous cacna1ab*tb204a-/-* larvae were viable and had reduced motility. Moreover, there was an increased incidence of synaptic failure at the NMJ due to reduced CaV2+ flux into the presynaptic NMJ, as detected by imaging of presynaptic intracellular CaV2+ (Wen et al., 2013). While this defect accurately predicted reduced motor function, neither sensory nor central effects of the mutation were assessed so their potential contribution to the behavioral phenotype cannot be excluded. Interestingly, both swimming behavior and NMJ synaptic transmission were rescued in cacna1ab*cacna1ab-/-* larvae by 3,4-diaminopyridine (a K+ channel blocker) and Roscovitine (a P/Q-type channel agonist; Yan et al., 2002; Buraei et al., 2007; Tarr et al., 2013).

The *fakir* cacna1ab mutation results in a L356V substitution in the S6 helix of Repeat I (Figure 1). Like the *tb204a* larvae, *fakir* mutants display reduced locomotor behavior compared to wild-type siblings. In addition, heterologously expressed *fakir* and *tb204* mutant channels had reductions in current amplitude and similar depolarizing shifts in channel activation properties (Low et al., 2012; Wen et al., 2013). *a priori*, L356V would appear to be a conservative amino acid change. However, L356 (located at the cytoplasmic side of S6 in RI) is highly conserved across species. Interestingly, the *tb204a* mutation (Y1662N) resides in an analogous location in S6 of RIV. While no disease-causing mutations have yet been identified in RIS6, human pathogenic point mutations were detected in the S6 helices of Repeats II–IV (Figures 1–3). Two of the mutations in S6 domains, V1494I and I1811L, would, similar to *fakir*, also be considered to be conservative substitutions. Overall, despite the identification of several S6 mutations, how L356V or other S6 mutations lead to perturbed channel function remains unknown. However, the fact that this is a highly conserved region across species suggests that mutations, even conservative ones, would be of consequence.

Despite the somewhat similar effects on channel activity produced by the two different cacna1ab mutations, substantially different mechanisms were proposed for how channel dysfunction leads to abnormal locomotor behavior. Consistent with the behavioral immotility, Low et al. (2012) found that rigorous swimming could be evoked in wild-type, but not *fakir* mutant, slow-twitch muscle by tactile stimulation. However, examination of responses to direct application of acetylcholine as well as miniature end plate current properties revealed little differences in transmission between motor neurons and slow-twitch fibers in *fakir* vs. wild-type larvae, nor were defects detected in evoked transmission between CaP motor neuron and fast-twitch muscle fibers. On this basis and consistent with the initial identification of *fakir* as a reduced touch-sensitive mutant (Granato et al., 1996), Low et al. (2012) proposed that *fakir* mutants have defective swimming responses to tactile stimulation because the relevant sensory neuron Rohon–Beard cell required cacna1ab for function. However, this hypothesis was not tested directly by recording from Rohon–Beard neurons or their pre-synaptic partners. In contrast, a study of the *tb204* allele provided strong evidence to support defective transmission at the NMJ (Wen et al., 2013). Supporting evidence was provided by paired recordings between one type of motor neuron, CaP, and its fast-muscle target cell. Whether similar transmission...
defects occur at the NMJs formed between other motor neurons and muscle targets has not been studied. Thus, the mechanistic bases for the reduced motility defects of fakir and tb204a mutants have not been resolved.

Despite this impasse, the viability of both the fakir and the tb204 mutant lines bodes well for the potential usefulness of zebrafish larvae carrying missense mutations corresponding to those which cause severe human Cav2.1 channelopathies (e.g., Cav2.1 R1673P). The generation of such models through CRISPR-Cas9 technology would enable the study of individual mutations with approaches encompassing the molecular, systemic, and behavioral levels. In particular, via paired CaP motor neuron—muscle recordings and imaging of depolarization-induced Ca$^{2+}$ flux into presynaptic terminals allow assessment of whether impairments in locomotor function result from NMJ defects.

Since zebrafish were successfully used to screen for compounds for the treatment of Dravet syndrome, a SCN1A\(\alpha^+\) channelopathy (Griffin et al., 2017), one can envision that this approach could be used to identify and/or refine small molecules to combat both Cav2.1 gain- and loss-of-function disorders. Compounds that partially counteract channel gain of function, notably gabapentin and pregabalin, were available for clinical use for sometime (Sills, 2006). However, a need for alternatives arose as both the aforementioned compounds were shown to have some addictive capability (Bonnet et al., 2018; Althobaiti et al., 2019). In regard to loss-of-function disorders, 3,4-diaminopyridine was approved for acute treatment of Lambert—Eaton syndrome, a condition secondary to an aggressive lung cancer in which autoantibodies to Cav2.1 are generated (Garcia and Beam, 1996; Maddison, 2012). Unfortunately, the arrhythmogenic potential of this compound precludes its long-term use in other contexts including the neurodevelopmental disorders discussed above. By contrast, derivatives of Roscovitine, such as those pioneered by the Meriney group, are logical candidates for further development (Tarr et al., 2013; Wu et al., 2018). Another possibility, which may not be a stretch given nascent cryo-EM images and the increasingly frequent implementation of deep learning approaches, is the modification of the L-type channel agonist (-)Bay K 8644 for use as a specific P/Q-type channel agonist (Zhao et al., 2019).
Despite these advantages, the zebrafish model system does pose some challenges. The fact that gene duplication endowed teleosts with two cacna1a genes can be problematic, even though the characterization of the tb204a mutant revealed that cacna1aa channel isoform makes little, if any, contribution to neurotransmission at the NMJ (Wen et al., 2013). However, sequence similarity between the isoforms may complicate knockdown experiments using antisense strategies and the production of reliable antibodies. Finally, zebrafish, like flies and mice, are not human. Nonetheless, the flexibility of the fish model makes it potentially useful as a first-line indicator of individual mutations and a vehicle for the development of personalized therapies.

CONCLUSIONS

Whole-exome sequencing is bringing new Cav-2.1 mutations out of the woodwork (see Damaj et al., 2015; Jiang et al., 2016; Weyhrauch et al., 2016; Luo et al., 2017; Travaglini et al., 2017). Many of the syndromes caused by these point mutations are more severe than the typical EA2 and FHM1 in that they present with not only ataxia or migraine but also with neurodevelopmental delay, nystagmus, epilepsy, cerebellar degeneration, hypotonia, and cognitive dysfunction. Modeling these more severe disorders is problematic because of the heterogeneous effects on channel function and the limitations intrinsic to flies and mice. Although not without some disadvantages, zebrafish present a useful model system for the timely characterization of pathological phenotypes and pharmacological correction.

AUTHOR CONTRIBUTIONS

ST, AR, and RB wrote the article. All authors read and approved the final manuscript.

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**Conflict of Interest**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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