Chapter 4

Genetic Defects of Voltage-Gated Sodium Channel α Subunit 1 in Dravet Syndrome and the Patients' Response to Antiepileptic Drugs

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Additional information is available at the end of the chapter

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Abstract

In the past decade, hundreds of mutations have been found in the *SCN1A* (sodium voltage-gated channel *α* subunit 1) gene in the epileptic patients. The functioning of the *SCN1A* gene products is intensively studied in the neuroscience field. The loss-of-function mutations of the *SCN1A* gene are the causative factor of Dravet syndrome, an intractable epilepsy syndrome. With the loss-of-function $Na_v1.1$ (the protein encoded by *SCN1A* gene), the selective dysfunction of the inhibitory parvalbumin (PV) interneurons impairs the balance of excitatory and inhibitory synaptic inputs to the downstream neurons, and causes the hyperexcitability of the neuronal network. The underlying mechanism is that the axon initial segments (AISs) of inhibitory parvalbumin interneurons predominantly express $Na_v1.1$, particularly in the proximal end of the AISs. The deficiency of $Na_v1.1$ weakens the excitability of the inhibitory parvalbumin neurons and leads to the hyperexcitability of the neuronal network. The sodium channel blockers, one category of the antiepileptic drugs (AEDs) that specifically block the activity of VGSCs, may potentially worsen the defect of $Na_v1.1$ of the PV interneurons in the patients with the *SCN1A* gene loss-of-function mutations, aggravate the clinical manifestation, and increase the seizure frequency of those patients.

Keywords: epilepsy, Dravet syndrome, voltage-gated sodium channel, axon initial segment, interneuron

1. Introduction

Voltage-gated sodium channels (VGSCs) play an essential role in the generation of the action potentials, which are the primary way for the communication between the excitable cells,

particularly the neurons. The action potential is the fast method to collect the afferent sensory information and to relay the efferent motor commands in the nervous system. The pathways to transfer the action potentials rely on the organized expression and the proper functioning of VGSCs [1]. The genetic mutations that cause the defected expression of VGSCs or the malfunction of the altered VGSC gene products impair the physiological function of conduction pathway [2], nerve nuclei [3], and cortical neurons [4]. Epilepsy is a common multifactorial neurological disease that is caused by both environmental and genetic factors [5]. Several ion-channel genes are evidently associated with epilepsy, such as *SCN1A* [6], *SCN2A* (sodium voltage-gated channel α subunit 2) [7], *SCN8A* (sodium voltage-gated channel α subunit 8) [8], *GABRA1* (gamma-aminobutyric acid type A receptor α 1 subunit), *GABRG2* (gamma-aminobutyric acid type A receptor γ2 subunit) [9], and *KCNA2* (potassium voltage-gated channel subfamily A member 2) [10]. Those genes predominantly control at least one critical event in a specific neuron type or/and a particular subcellular region during the physiological functioning of the neurons. The irreversible dysfunction of those gene products leads to the permanent pathological alteration of the targeted neurons and increases the susceptibility to seizures.

2. VGSC α subunit 1 and Dravet syndrome

$Na_v1.1$ existing in the majority in the brain and was labeled at the soma and dendrites of the neurons in the early studies, referred as Na + channel subtype RI, or Type I Na(+) channel alpha-subunit [11, 12]. Nowadays, more than 1000 mutations [13] have been found in the *SCN1A* genes. They have been believed as the causative factors of generalized epilepsy with febrile seizure plus (GEFS+) [14], Dravet syndrome (severe myoclonic epilepsy of infancy—SMEI) [15], and migraine [16]. The *SCN1A* gene could be called as "an epilepsy gene" because of its close relationship with febrile seizures and the epilepsies with antecedent febrile seizures [13]. Dravet syndrome has been intensively studied within *SCN1A* gene mutations, the related functional analysis [6, 17], and the high phenotype-genotype correlations to *SCN1A* gene [18]. It is a rare disease of 1-to-40,000 incidence in the USA at estimate and 1-per-15,700 incidence in northern California with evidence [19]. The debilitating clinical progress goes from the "febrile stage" in the first year of life, the "worsening stage" until the age of 6 years, to the "stabilization stage" in the rest of life [20]. The clinical case may have the family history of epilepsy or febrile seizures, no previous medical history (an apparently normal baby), and the generalized or unilateral febrile seizures beginning in the first year followed by myoclonic jerks, partial seizures, atypical absence, and status epilepticus. The patients progressively lose the neurological functioning, such as retarded psychomotor development, ataxia, pyramidal signs, interictal myoclonus, intellectual deficiency or cognitive impairment, and personality disorders [21]. A French pediatrician, Dr. Charlotte Dravet, first described the rare disease in 1978 [22], and in 2001, Lieve Claes team in Belgium found seven *SCN1A* gene mutations in SMEI patients, including four frameshift and truncation mutations (c.657-658delAG, c.3299-3300insAA, c.5010-5013delGTTT, and c.5536-5539delAAAC), one nonsense mutation (c.664C>T), one splice donor mutation

(IVS22+1G>A), and one missense mutation (c.2956C>T) [15]. The subsequent genotyping studies indicated that the *SCN1A* gene truncation mutations appeared with the higher frequency in Dravet syndrome than in the milder phenotype of febrile seizure-related epilepsy, and furthermore, the cognitive function impairment of the *SCN1A*-positive epilepsy patients also associated with the truncation variants, regardless of age at seizure onset [23]. Those truncation mutations severely alter the molecular structure of sodium channel, reduce the expression amount of the *SCN1A* gene products, and hence severely impact the $Na_v1.1$-VGSC functioning in the neurons. The underlying pathogenic mechanism of one-allele truncation could be due to the haploinsufficiency (the loss of the half amount of $Na_v1.1$) [24] or a dominant-negative effect that represents the mutated proteins (truncated $Na_v1.1$) negatively affect wild-type products (normal allele-expressed $Na_v1.1$) [25]. However, the truncated $Na_v1.1$ protein in the human brain sample of a patient with Dravet syndrome was not detected, while the mRNA of both the wild-type and the truncation mutations was equally expressed. It was explained as the endoplasmic reticulum-associated degradation of the misfolded or misassembled protein caused the absence of the truncated $Na_v1.1$ protein [26]. Therefore, the lower expression of the $Na_v1.1$ protein, the haploinsufficiency of *SCN1A* gene, should be the main pathogenesis of *SCN1A* truncation mutations. The remaining mutations of *SCN1A* gene causing Dravet syndrome are the missense and splice-site mutations that mistakenly code the amino acids building the pore-forming region of VGSC [27] and cause the severe impairment of the function and electrophysiological properties of the $Na_v1.1$ protein (VGSC α subunit 1) [28–30].

Both the haploinsufficiency and the pore-forming mutations of *SCN1A* gene impact the VGSC function severely because structurally the $Na_v1.1$ protein constructs the main part of VGSC complex. This type of sodium channel is made up of a large pore-forming glycosylated α subunit and one or two small β subunits non-covalently ($\beta1$ or $\beta3$) or covalently ($\beta2$ or $\beta4$) associated with the large α subunit. The α subunit is coded by *SCN1A-SCN11A* gene ($Na_v1.1$-$Na_v1.9$ protein) and responsible for the generation of transmembrane sodium current. The β subunits regulate the electrophysiological properties of sodium current (the gating and kinetics of α subunits) and the subcellular location or expression of α subunit [31]. Those mutations disable the main part of VGSC, α subunit, by either the insufficient amount of subunit for the complex resembling or the structurally altered subunit to make the malfunctioning complex. The primary structure of the VGSC α subunit contains four internally repeated transmembrane domains (I–IV). Each domain includes six α-helical transmembrane segments (S1–S6). The S1–S4 segments are the voltage-sensing module, and the S5–S6 segments are the pore-forming module (D400, E755, K1237, and A1529 in each of four homologous domains, forming part of the selectivity filter [32]). Nine members of VGSC α subunits ($Na_v1.1$–$Na_v1.9$) have been identified with similar complex structure and functional properties [33]. $Na_v1.1$ is tetrodotoxin (TTX)-sensitive and could be blocked by nanomolar concentrations of TTX [34]. $Na_v1.1$, $Na_v1.2$ (*SCN2A*), $Na_v1.3$ (*SCN3A*), and $Na_v1.6$ (*SCN8A*) primarily locate in central nervous system and are the primary subtypes of VGSCs in the neurons of brain and spinal cord [35]. Four subtypes of VGSC α subunits in the central nervous system express in the specific neuron types and the specific subcellular location [36, 37].

3. Animal models and pathogenic mechanism of Dravet syndrome

Based on the genotyping results that the *SCN1A*-gene truncation mutations are found in the large percentage of patients with Dravet syndrome [18], the scientists produce the genetic models to disrupt the *SCN1A* gene by truncation mutations. They successfully duplicate the clinical features in mouse models of Dravet syndrome, such as spontaneous and frequent seizures, impaired neurological function, and premature death [4, 38, 39]. Yu et al. at the University of Washington, USA, first replaced the last exon (Exon 26) of *SCN1A* gene of 129/SvJ and C57BL/6 mice with neomycin-resistance gene cassette to make the truncation at the domain IV, S3 segment of $Na_v1.1$ protein. By measuring whole-cell sodium currents of the dissociated neurons, the authors found a substantial decrease in the sodium current amplitude and other significant alteration of electrophysiological features, which indicated the reduced amount of the functional VGSCs in *Scn1a*−/− and *Scn1a*+/− interneurons. They also confirmed that in those GABAergic inhibitory interneurons the VGSC subtype $Na_v1.3$ was upregulated to compensate the reduced functional $Na_v1.1$ protein, without the apparent increase of $Na_v1.2$ and $Na_v1.6$ [4]. The group of Mistry at the Vanderbilt University, USA, generated another *SCN1A* gene truncation mice in 2014 by disrupting Exon 1 of the *SCN1A* gene. They measured the electrophysiological features of acutely dissociated hippocampal neurons and found that the sodium channel density was lower in GABAergic interneurons of the *Scn1a*+/− mice. Furthermore, the sodium channel density in excitatory pyramidal neurons of *Scn1a*+/− mice was also elevated, which potentially correlated with age-dependent lethality. Although the phenotype severity was variable due to the factors of strain and age of mice with Dravet syndrome, the findings first emphasized the contribution of pyramidal neuron hyperexcitability during the pathological process in the study of animal model [38]. Both of the studies point out that the imbalance of electrophysiological activities of the excitatory (pyramidal neurons) and the inhibitory neurons (GABAergic interneurons) is the fundamental pathogenesis to cause the intractable seizures in mice with Dravet syndrome that had the *SCN1A* gene truncation mutations.

Furthermore, the Ogiwara group in Japan provided the results from an *SCN1A* truncation knock-in mouse model, which displayed the specific subcellular region of $Na_v1.1$ deficiency in those dysfunctional inhibitory neurons. By inserting the nonsense mutation R1407X into mouse *SCN1A* gene, the authors found that the truncated $Na_v1.1$ protein was not detectable in either $Scn1a^{RX/RX}$ or $Scn1a^{+/RX}$ knock-in mice. In the neocortex of those developing mouse brain, only a subpopulation of the neocortical neurons, the parvalbumin-positive interneurons (PV neurons), had the $Na_v1.1$ immunostaining signals, predominantly at the axon initial segments (AISs). Consequently, the $Scn1a^{+/RX}$ neocortical PV interneuron (GAD67+), fast-spiking interneurons, displayed the spike amplitude decrement during prolonged spike trains [6, 39]. That important study proved the specific functional defect of the PV neurons and the critical pathophysiological role of the PV neurons in Dravet syndrome. Moreover, in an in-vivo animal study, using the advanced techniques, such as optogenetics, local field potential, and multiunit activity signals recording, the authors surprisingly found that the spontaneous cortical activity of *Scn1a*+/− mice did not alter in vivo. Although after sacrificing those mice, they could recognize the seizure-related pathological changes in the brain slices, such as the hypoexcitability of the parvalbumin and somatostatin interneurons, the rapid propagation of epileptiform activity, and the pathogenic synaptic adaptions [40]. Therefore, we could reason

that the interneuron hypoexcitability should exist much earlier than the electroencephalography (EEG)-positive findings in the patients with Dravet syndrome.

The other type of *SCN1A* gene mutations causing Dravet syndrome is the missense mutation in the pore-forming region of VGSC. Another group in Japan, using gene-driven ENU mutagenesis technique, generated *Scn1a*-targeted rats carrying a missense mutation N1417H miscoding the amino acid in the third pore region of VGSC [29]. The clinical feature of the N1417H rat was milder than that of truncation *Scn1a* mouse models. The rat had neither spontaneous seizures nor apparent pathological abnormality in the brain in the earlier life, but at postnatal week 5, after a hot water bath about 3.5 min, the rats exhibited clonic seizures. The susceptibility to hyperthermia-induced seizures increased with age. The hippocampal GABAergic interneurons of the N1417H rats were hypoexcitable with the reduced action potential amplitude. The authors believed that the clinical phenotype of the N1417H rat was close to that of generalized epilepsy with febrile seizure plus (GEFS+) [41]. However, the common pathogenic mechanism of the truncation mutations and the pore-forming region mutations of $Na_v1.1$ is the selective interneuron dysfunction and hypoexcitability due to the $Na_v1.1$ deficiency or malfunction. The results from the study of the N1417H rat enhance the hypothesis that the selective impairment of the PV interneurons is the primary pathogenesis of febrile seizure–related epilepsy syndrome, the clinical spectrum from GEFS+ to Dravet syndrome [18].

4. Inhibitory interneurons and the features for their specialties

During the processing of the neuronal activities in the central nervous system, the simultaneous excitation and inhibition assure the proper excitability of the neuronal network and the precise control of the neuronal functions. Inhibition in the cortex is generated by the GABAergic neurons, which make up about 20% of the cortical neuronal population. Compared with the pyramidal cells (excitatory neurons), they have the smaller size and much shorter-range projects of the axon to form the local circuit with the nearby neurons and layers [42]. The interneurons could generate the long-lasting currents, the faster reaction to stimuli, and the higher-frequency signal transmission. The inhibition in a neuronal microcircuit could apply at the right millisecond (timing) and with the precise amount (dosing) exactly matching the inhibitory demand [42, 43]. The defects of GABAergic neuronal function have been identified as the contributive factors to the neuronal diseases, such as epilepsy, schizophrenia, and autism spectrum disorders [44, 45]. The cell therapy strategy of the GABAergic neurons for epilepsy was applied in several significant studies of epileptic model and stem cells [44, 46]. In the studies of animal models of Dravet syndrome, the constitutive Na1.1 knockout selectively impacted the functioning of the inhibitory parvalbumin interneurons, spared the detectable dysfunction of the excitatory neurons, and caused by the imbalance of excitation and inhibition, which led to the spontaneous and intractable seizures [4, 38, 39]. On the background of the $Na_v1.1$ knockout specifically in the PV neurons, the addition of $Na_v1.1$ knockout specifically in the excitatory neurons could alleviate the clinical manifestation of Dravet syndrome [6] and potentially re-balance the excitatory and inhibitory neuronal activity. Based on those results, we can understand that the balancing status of inhibitory neurons and excitatory neurons functioning is the determinant of the clinical phenotypes of Drave syndrome.

The several types of inhibitory interneurons are called as "basket" cells, "chandelier" cells, and "Martinotti" cells due to their morphological features. Because of their morphological advantages, they connect and inhibit the particular compartment of principal neurons [42]. The "basket" cells have the highly branched axons that innervate the target somas and the proximal dendrites of pyramidal neurons, as the axonal branches appear like baskets surrounding the pyramidal neurons. In hippocampus CA1, the parvalbumin-expressing basket cells (PVBCs, 26% of CA1 interneurons) are more than the cholecystokinin-expressing basket cells (CCKBCs, 12% of CA1 interneurons) [47]. The PVBCs place 99% output to connect the pyramidal cells and the rest 1% output to form the gap junctions and reciprocal synaptic connections onto themselves or other interneurons generating gamma oscillation [48, 49]. The "chandelier" cells that are also parvalbumin positive have the "cartridges" shape of the axonal arbors that selectively inhibit the AISs of pyramidal cells, and hence they are also called the axon-axonic cells providing precise control of the action potential generation of pyramidal cells [48, 50, 51]. The axon-axonic cells represent about 15% of all PV hippocampal interneurons [47]. The "Martinotti" cells target the apical dendritic tuft and express the somatostatin and calbindin but not parvalbumin or vasoactive intestinal peptide (VIP) [52]. In the hippocampus, the rest of PV cells is "Bistratified" cells, which represent about 25% of PV hippocampal interneurons with the PV-immunosignal on the somatodendritic compartments. In the PVBCs, the Na^+ channels are sparse in the dendrites where K^+ channels predominate. The Na^+ channels cluster at the AIS of PVBCs. In fact, 99% of PVBC Na^+ channels are located in the axonal compartment [53]. The unique feature of the high-density distribution of Na^+ channels at the PVBC AISs determines the fast-spiking pattern of the PVBCs, which typically generate uniform, non-changing, and high-frequency discharge [54].

5. AIS and VGSC

Axon initial segment (AIS) contains the high density of sodium and potassium channels; the scaffolding protein ankyrin G (AnkG), βIV spectrin, and extracellular matrix–binding protein neurofascin; and the ion channel–associated protein FGF14 (fibroblast growth factor 14). Those are necessary to help the sodium channels locate and cluster at the AIS [55]. The AIS has the lowest threshold for action potential initiation because of the highest density of sodium channels when compared with somatodendritic compartment [53]. The proper functioning of AIS is essential for action potential initiation and adaptive cell excitability of both pyramidal cells (excitatory neurons) and GABAergic interneurons (inhibitory neurons). Many factors regulate the function of the sodium channels at the AIS [55, 56]. First, the distinctive VGSC α subunit types express in specific neuronal types and the particular regions of AIS. In the human brain tissue, the fluorescence signals of $Na_v1.1$ have been found at the thinly AnkG-labeled AIS, which putatively belongs to the interneurons, while $Na_v1.2$ and $Na_v1.6$ are located at the AISs of human cortical pyramidal cells [51]. $Na_v1.6$, the low-threshold sodium channel subtype, accumulates at the distal end of AIS of cortical pyramidal cells, which is responsible for generating the action potentials. The high-threshold $Na_v1.2$ locates at the proximal end of AIS of cortical pyramidal cells, which regulates the action potential backpropagation [57]. The

$Na_v1.1$ has been found at the proximal end of AIS of cortical and cerebellar interneurons and the axons of main olfactory bulb neurons. The $Na_v1.1$ immunosignals predominantly outline the axons of the parvalbumin-positive neurons [58]. The action potential threshold of $Na_v1.6$ is more hyperpolarized (15–25 mV lower) than that of both $Na_v1.2$ and $Na_v1.1$. Unlike $Na_v1.6$ more likely producing a persistent current, $Na_v1.1$ and $Na_v1.2$ show the apparent use-dependent inactivation (higher than 20 Hz) [59, 60]. Therefore, the accumulated $Na_v1.6$ at the distal end of the AIS facilitates the action potential initiation, while $Na_v1.2$ and $Na_v1.1$ subunits gathering at the proximal end of AIS prevent the high-frequency firing of nerve cells backward.

Second, the molecule complex at AIS, composed of the ion channels (Kv1, T-type Ca^{2+} channel) and ligands (FGF14, VGSC β subunit 1, and βIV spectrin), directly or indirectly cooperates with Na^+ channel and regulates the neuronal excitability. FGF14 could directly interact with the C-terminal of VGSC α subunit ($Na_v1.1$, $Na_v1.2$, $Na_v1.6$) in the transfected HEK293 cells [61]. The *fgf14*−/− mice showed the significantly reduced number of the PV interneurons, but the pyramidal neuron number was unchanged in the CA1 hippocampus region. This change cooperated with the reduced GAD67 immunosignals in PV cell soma, the reduced gamma oscillations in CA1 stratum radiatum layer, and the deficits in spatial working memory, which was displayed by the eight-arm maze test [62]. Because the FGF14 is a complementary protein of $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ at the AIS [61, 63, 64], the pyramidal cells of the *fgf14*−/− mice should have been affected as well due to the loss of FGF14:$Na_v1.6$ and FGF14:$Na_v1.2$ complex. Why are the PV cells the first or most to be affected by FGF14 protein defect or the FGF14:$Na_v1.1$ complex deficiency? What makes the FGF14:$Na_v1.1$ critical for the excitability of the PV cells and finally impact on the number of the live PV cells? Similarly, the $Na_v1.1$-predominant proximal end of the AIS only occupies a small part of the AIS of the PV cells, and why the $Na_v1.1$ deficiency impairs the PV cell functions dramatically and causes the Dravet syndrome? We expect the promising studies and explanation in future. Other molecules in the AIS complex, such as VGSC β subunit and AnkG, have been recognized as the ligand or the anchoring protein to stabilize the VGSC α subunit and regulate their functions at the AIS [65, 66].

Third, the location and the size of the AIS can be adapted for the neuronal activity and the long-term plasticity. The longer AIS, the higher excitability of the neuron. The more proximal location of the AIS, the higher excitability of the neuron. The chronic depolarization of the dissociated neurons moved the AIS distally and then decreased the neuronal excitability. The dynamic regulation of the AIS location through activity-dependent structural reorganization relied on the activation of T-type voltage-gated calcium channels and the elevation of intracellular [Ca^{2+}] [67]. On the other hand, the experiments to eliminate the sensory stimuli made the AIS longer with little change in Na^+ channel density and ion channel composition at the AIS, which increased the whole cell Na^+ current, and the neuronal excitability. However, those adaptive responses also depend on the neuronal types due to the distinctions of the AIS location of different neurons under the standard conditions [67]. In the PV interneurons, the action potentials are generated at 20 μm away from the soma at the AIS [53], which means the proximal part of the AIS of the PV neurons locates even closer than 20 μm since the action potential generates at the beginning of the distal part of the AIS. The AIS of the PV neurons locates more proximal than the AIS of pyramidal cells that has been observed at 20–60 μm from the soma by ankyrin G staining [68]. Using specific neuronal marker labeling,

Höfflin et al. saw the AISs of the pyramidal neurons were significantly longer than that of the interneurons [69]. Based on those findings of the PV interneurons and their AISs, neuroscientists may have many interests in the regulatory mechanisms of $Na_v1.1$ cooperative functioning and adaptive response to the neuronal activity, coupling with the dynamic plasticity of the PV interneuron AIS.

With the specialized output structures of PV interneurons ("basket" or "chandelier cartridge"), the interactions of the PV interneurons (PVBCs or chandelier cells) and the pyramidal cells, inhibitory synapses, are accordingly subject to the dynamic regulation of adaptive neuronal functioning and the AIS plasticity. The chandelier cell axon terminals only contact the AISs of pyramidal cells and have three to five boutons per cartridge. The innervation patterns are similar at different postnatal age. Multiple chandelier cells (four at estimate) connect one pyramidal cell, while one chandelier cell contacts 35–50% of pyramidal cells in the traversed area by its axonal arbor [70]. The inhibitory synapses exist in the innervation of a chandelier cell to the pyramidal cell by nature. The innervation could be visible by labeling the chandelier cells (pre-synaptic component) with the marker of GABA membrane transporter 1 (GAT1) or parvalbumin (PV) and labeling the post-synaptic pyramidal cell AIS with $GABA_A$ receptor α_2 subunit. The structures and functioning status of those synapses keep updated to meet the dynamic developmental demands [71, 72] and are impacted in the specific areas by pathological conditions, such as epilepsy and schizophrenia [73, 74].

6. Treatment to Dravet syndrome and therapeutic response

Dravet syndrome is an intractable epileptic encephalopathy with the unfavorable outcome. The most commonly used AEDs for patients with Dravet syndrome include valproate, topiramate, benzodiazepines, stiripentol, and potassium bromide [22]. Because the high percentage of patients with Dravet syndrome have the *SCN1A* gene mutations [18], some AEDs should be avoided, such as the sodium channel blockers (lamotrigine [75] and carbamazepine [76]), to prevent the inhibition of the rest $Na_v1.1$ functioning in PV interneurons. Guerrini et al. retrospectively reviewed 21 Dravet syndrome cases and found that 80% (17) patients with lamotrigine treatment showed >50% increase in seizure frequency for 2 months and then ceased the lamotrigine treatment [75]. In a large-sized (276 patients) study, Shi et al. showed the evidence that the treatment of carbamazepine to Dravet syndrome was either not effective (<50% seizure reduction) or worsening the clinical condition (>25% increase in seizure frequency) [76]. Considering the high percentage of *SCN1A* gene mutations in Dravet syndrome and the potential risk of worsening seizures by AEDs, the clinicians recommend a screening test to detect the *SCN1A* gene mutations for the suspected patients with Dravet syndrome (the initially normal infants suffering from prolonged, recurrent, febrile, and hemiclonic seizures induced by bathing). The purpose of the screening test is to optimize AEDs and rehabilitation therapy [77, 78] at the earlier stage of the disease. The test can be done with the direct sequencing of the coding exons of *SCN1A* gene or multiplex ligation-dependent probe amplification [79]. There are an increasing number of the adult patients with the intractable seizures, who finally are diagnosed with Dravet syndrome. They may retain the partial seizures,

secondary generalized clonic-tonic seizures [80], and intellectual disability, which are all not the characteristic symptoms for the diagnosis of Dravet syndrome. It is not occasional that those underdiagnosed patients with Dravet syndrome have been treated with sodium channel blockers, such as carbamazepine and oxcarbazepine, for years. However, the attempt to withdraw the treatment of carbamazepine or oxcarbazepine from those adult patients is still risky for seizure frequency rebound, withdrawal-related seizure, and sudden death [81].

Valproate is the most frequently used AED to treat Dravet syndrome. Shi et al. found that 87% of *SCN1A* mutation patients and 78% of *SCN1A* negative patients were using valproate. About 52% of *SCN1A* mutation patients and 41% *SCN1A* negative patients responded to the valproate treatment (>50% reduction in seizure frequency). Bromide (potassium bromide and sodium bromide) was the most effective AED. About 41% *SCN1A* mutation patients and 21% *SCN1A* negative patients used bromide with 71% and 94% responder rate, respectively. The *SCN1A* negative patients had the significantly higher responder rate to bromide, when compared with *SCN1A* mutation patients [76]. Bromide therapy was usually used in combination with valproate (100%), topiramate (91%), clobazam (75%), levetiracetam (66%), and so on. After 3 months of treatment, 81% of patients (26/32) had >50% reduction in seizure frequency, and after 12 months of treatment, 47% of patients (15/32) still maintained >50% seizure reduction [82]. Verapamil add-on treatment was reported in two pediatric patients with Dravet syndrome. They gained the long-period (13 months and 20 months) seizure free with the verapamil dosage of 1.5 mg/kg/day. As a result of a long period of seizure free, the patient's neurological and cognitive function improved significantly [83]. Levetiracetam add-on treatment was proved as an effective therapy to Dravet syndrome by an open-label clinical trial. The dose was titrated up to 50–60 mg/kg/day within 5- to 6-week up-titration phase and maintained in a 12-week evaluation phase. The responder rate ranged from 44.4 to 64.2% varied by the distinctive seizure types [84]. Stiripentol is one of the commonly used medications for Dravet syndrome. Because of its fair tolerability profile, stiripentol is frequently added within valproate and clobazam and maintained in the triple long-term therapy remedy (96% patients). However, the effectiveness of stiripentol is not ideal for seizure control. De Liso et al. evaluated 54 patients with Dravet syndrome after stiripentol maintenance at the dose of 35–50 mg/kg/day. They found that 96% of patients continued to have clonic or tonic-clonic seizures (38% of patients had the seizure frequency more than three times per month, 40% of patients remained seizure frequency once to three times per month, and the rest of patients remained yearly seizures) [85]. Balestrini and Sisodiya observed stiripentol add-on treatment in 13 patients with Dravet syndrome. Only 23% of patients (3/13) gained >50% reduction in seizure frequency, 23% of patients (3/13) showed seizure worsening, 23% of patients (3/13) showed no change, and 15% of patients (2/13) showed <50% reduction in seizure frequency [86]. Obviously, the responder rate or effectiveness of the pharmacological treatment of Dravet syndrome is not ideal. The clinicians also assessed the vagus nerve stimulation for Dravet syndrome treatment. Fulton et al. placed the vagus nerve stimulation for 12 patients with Dravet syndrome and assessed the seizure control after 6 months. Nine of them showed >50% reduction in generalized tonic-clonic seizures, and four of them showed the cognitive function improvement [87]. Neuroscientist screened the potential therapeutic agents and tested their effectiveness in vivo in the experimental setting. *Scn1Lab* zebrafish model was

used for the fast drug screening for *SCN1A*-mutated Dravet syndrome. Baraban et al. applied the molecules in the swimming bath of the mutant or control zebrafish, tracked the swimming velocity and behaviors of the zebrafish, and identified clemizole that could inhibit seizure behavior and electrographic seizures of the mutant zebrafish [88]. Ohmori et al. found that the intraperitoneal methylphenidate could improve the behavior (hyperactivity, anxiety-like behavior, and spatial learning impairments) of N1417H-*Scn1a* mice and significantly suppress the hot bath-induced seizures [89]. Similar to the results from human case studies, the N1417H-*Scn1a* mice showed the good therapeutic response to potassium bromide (reduced seizure duration) without significant impairment in motor coordination [90].

7. Discussion

Epilepsy is a chronic neurological disease worldwide, which jeopardizes the patients' lives, burdens the patients' family and caregivers, and requires to be concerned with the increasing attention to the affordable therapies, the effectiveness of current treatment strategies, and the social support to the patients and the caregivers. The pharmaceutical therapy (the application of AEDs) is the most commonly used strategy to fight against epilepsy. However, 30% of epilepsy patients are resistant to the optimized AED treatment without obvious precipitating factors [95]. Dravet syndrome might be an intractable and adverse form of extremity, which requires the multiple AED remedy (**Table 1**) and resistant to many AEDs over time [22]. The two major mechanisms are responsible for the resistance to AEDs in chronic epilepsy. One is the desensitization or modification of the molecular targets of AEDs during the chronic pathological process (frequent and recurring seizures), and the other is the overexpression of multidrug transporters, such as P-glycoprotein [96]. The sodium channel blocker is one of the main classes of AEDs. The genetic polymorphisms of the molecular targets, VGSC α subunits, are significantly associated with AED resistance. The genetic variant of *SCN2A* IVS7-32A>G (rs2304016) did not alter the *SCN2A* mRNA expression quantity or the exon splicing but had statistically significant association with AED resistance [97]. The calcium channel is also the therapeutic target of some AEDs, such as retigabine and ethosuximide (ETS). The genetic association study of drug-resistant epilepsy in the Chinese Han population displayed that the specific haplotype of the gene-coding calcium voltage-gated channel subunit alpha1 A (*CACNA1A*) was the risk factor for AED resistance [98]. How do those polymorphisms facilitate the desensitization of the AED target? We still need the extended functional studies to reveal the underlying mechanism. For instance, a significant cell functional study of the human brain samples collected from the surgery of hippocampal sclerosis resection provided the evidence of one mechanism of AED resistance (the modification of the cellular target of AEDs). Some categories of AEDs (benzodiazepines and barbiturates) stimulate the gamma-aminobutyric acid ($GABA_A$) receptor and increase the intracellular concentration of chloride and synaptic inhibition. However, the expected GABAergic response of the treated pyramid cells relies on the proper functioning and the normal expression profile of chloride transporters (Na-K-2Cl cotransporter NKCCl and K-Cl cotransporter KCC2). Otherwise, the $GABA_A$ receptor activation depolarize the postsynaptic compartments, which contributes to the epileptiform activity in the stimulated pyramidal cells. In fact, in the temporal lobe epilepsy biopsy tissue, a small group of pyramidal cells downregulated the expression of KCC2 and

Agent or medication	Main action for treatment	Dosage in the treatment	Responder rate (>50% reduction)	Retain period (year)	Retain combination remedy	Aggravation rate (>25% increase)	Cause of death (SE or SUDEP*)
Valproate (VPA)	Na^+ Ca^+ channel ↓ GABA ↑ GLUT ↑ [91]	30–50 mg/kg/d [92]	52 and 41% [76]		VPA + Br VPA + CZP + Br VPA + CLB + Br [81]		SUDEP [92]
Topiramate (TPM)	Na^+ Ca^+ channel ↓ GABA ↑ GLUT ↑ [91]	7.5–15 mg/kg/d [92]	57 and 33% [76]			17% [82]	
Clobazam (CLB)	GABA ↑ [91]	0.2–1 mg/kg/d [92]	44 and 48% [76]		VPA + Br VPA + CLB + Br [81]		SUDEP [92]
Clonazepam (CZP)	GABA ↑ [91]	0.03–0.1 mg/kg/d [92]	44 and 38% [76]		VPA + Br VPA + CZP + Br [81]		
Zonisamide (ZNS)	Na^+ Ca^+ channel ↓ [91]		36 and 38% [76]			44% [82]	
Phenobarbital (PHB)	GABA ↑ [91]		29 and 35% [76]				
Phenytoin (PHT)	Sodium channel blocker [91]		10 and 29% [76]			50% [82]	
Stiripentol (STP)	GABAergic enhancer [93]	35–50 mg/kg/d [85]	23% [86]	5 [86]	VPA + CLB CLB + TPM VPA + TPM [86]	23% [86] 6% [82]	
Bromide (Br)	Stabilize the excitable membrane through hyperpolarization [94]	30–70 mg/kg/d [76] 30–106 mg/kg/d [82]	71 and 94% [76] 81% [82]	2.5 [76] 2 [82]	VPA + Br VPA + CZP + Br VPA + CLB + Br [76] VPA + TPM + CLB [82]		
Levetiracetam (LEV)	Act as a neuromodulator	50–60 mg/kg/d [84]	44–64% [84]		VPA + TPM + LEV [84] VPA + CLB + LEV [84]	5% [82]	
Lamotrigine (LTG)	Sodium channel blocker [91]	2.5–12.5 mg/kg/d [75]	5% (1/21) [75]	14 m# [75]	VPA + CZP + CLB [75]	57% [82] 80% [75]	SUDEP [92]

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Agent or medication	Main action for treatment	Dosage in the treatment	Responder rate (>50% reduction)	Retain period (year)	Retain combination remedy	Aggravation rate (>25% increase)	Cause of death (SE or SUDEP*)
Carbamazepine (CBZ)	Sodium channel blocker [91]	200–900 mg/day [81]	9% [76]	0.9–>20 [81]	VPA CLB STP ZNS LEV CLN TPM PHT [81]	33% [81] 71% [82] 21% [76]	SUDEP, SE [81]
Oxcarbazepine (OXC)	Sodium channel blocker [91]	600–1200 mg/day [81]	Withdrawal + complete stop	0.1–>20 [81]	VPA CBZ ZNS LEV CLN PHB [81]	72% [82]	SUDEP [81]
Verapamil	Voltage-gated calcium channel blocker [83]	1.5 mg/kg/d [83]	2/2 [83]	13–>20 m#	VPA TPM PHT ETS† [83]		
Clemizole	H1 antagonist [88]	100 μM swim bath (zebrafish)	Significant reduce seizure behavior	Single dose	Monotherapy [88]		
Methylphenidate	Increase dopamine release (rat) [89]	0.5–2.0 mg/kg i.p. [89]	Significant improvement	Single dose	Monotherapy [89]		

*SUDEP, sudden unexpected death in epilepsy; SE, status epilepticus.

#m: month.

†ETS, ethosuximide.

Table 1. The commonly used AEDs and the experimental therapies that have been applied in the treatment of Dravet syndrome.

led to depolarizing the postsynaptic neurons [99]. Because Dravet syndrome would be treated intensively with multiple AEDs for a long time, the multifaceted mechanisms might involve the AED resistance during the disease development. They could be the pathological alteration of the target cells at cellular/molecular level (downregulation of KCC2 in the pyramidal cells) or the overexpression of multidrug transporter genes that code ATP-binding cassette transporters 1–4 (*ABCB1-4*) in the epileptogenic zone (hippocampal sclerosis) [100]. The research interests might arise from those points in the future neuroscience and epileptology fields.

8. Conclusions

Dravet syndrome, an intractable epilepsy syndrome, affects the initially normal infants with febrile or non-febrile seizures, myoclonic seizures, hemiclonic seizures, and developmental delay. The *SCN1A* gene mutations are frequently found in patients with Dravet syndrome with "severe" genotypes, such as truncation or pore-region missense mutations. The *SCN1A* haplodeficiency mice display spontaneous seizures, cognitive impairment, and premature death, similar to human clinical phenotype of Dravet syndrome. The electrophysiological findings indicate that the parvalbumin-expressing interneurons in those mice are dysfunctional and hypoexcitable. The selective dysfunction of PV interneurons causes the imbalance of excitatory and inhibitory control to the principal neurons and neuronal network. The immunostaining has confirmed that $Na_v1.1$ highly expresses at the axon initial segment of PV interneurons. Due to the complex and dynamic plasticity of AIS and the adaptation response of ion channels to neuronal activity, the $Na_v1.1$ functioning within the AIS plasticity of PV interneurons and mutant pathogenesis remains unknown and would bring out the intensive studies in future. Patients with Dravet syndrome are always treated with multiple AEDs with disappointing outcome (**Table 1**). The most commonly used and retained AED in the treatment is valproate. The most evidently effective medication is bromide. With the aim to optimize the pharmacological treatment and encourage the earlier intervention to neurological development, the early genetic screening test of *SCN1A* gene is recommended to the possible patients with Dravet syndrome. Patients with Dravet syndrome should avoid the sodium channel blockers to prevent greater extent inhibition of $Na_v1.1$ function that potentially worsens the seizures. We expect that the more promising results would be generated in the experimental therapy studies of Dravet syndrome and provide the valuable resources to help the patients with Dravet syndrome and overcome the devastating disease.

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Conflict of interest

The author declares that there is no conflict of interest.

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