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CLINICAL STUDY

Phenotypic spectrum of the SCN1A mutation (from febrile seizures to Dravet syndrome)

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ABSTRACT
Dravet's syndrome – previously known as severe myoclonic epilepsy in infancy, is classified as epilepsy on a genetic basis (1). 70–80 % of the patients with the Dravet's syndrome phenotype are associated with the detection of a sequence variant in the SCN1A gene (alpha 1 subunit of the voltage-gated sodium channel) (2). However, sequence variants in the SCN1A gene are associated with a very broad clinical spectrum, from asymptomatic carriers to the severe myoclonic epilepsy phenotype with severe disease (3).
In the presented work, we retrospectively evaluated a group of 6 patients of the Department of Pediatric Neurology of the Medical Faculty of Masaryk University and the University Hospital in Brno with a proven missense mutation. Based on the specific pathogenic sequence variant, we correlated the patient's phenotype with the location of the sequence variant in the SCN1A gene. The aim of the analysis was to verify the extent, to which the storage of a pathogenic sequence variant in the SCN1A gene corresponds to the clinical picture of the patient *(Tab. 2, Fig. 2, Ref. 10)*. Text in PDF *www.elis.sk*
Key words: Dravet's syndrome, sodium channel, functional analysis, prognosis.

Introduction

The SCN1A gene encodes a voltage-gated channel which, due to its wide distribution in the central nervous system, plays an important role in controlling the excitability of neurons (4). Sequence variants in the SCN1A gene are associated with a broad phenotypic spectrum: epilepsy, autism, hemiplegic migraines. In the case of epilepsy, it is a spectrum from febrile convulsions, through the image of GEFS + (generalized epilepsy with febrile seizures) to the image of Dravet's syndrome. Cases of asymptomatic carriers are not uncommon (3). This fact therefore leads to the need to determine the pathogenicity of a given sequence variant (5).

The structure of the alpha one subunit of the sodium channel is well known. Functionally important areas are: pore area, voltage sensor area – so-called V-sensor (area S4 and adjacent areas), terminal areas (C and N terminal area), loops between domains (so-called D-linker) D1 and D2, D2a D3 and D3 and D4 as well as the region of the remaining S1–S3 subunits (3, 6). Due to the location of a particular sequence variant, its pathogenicity and clinical significance for the patient can be further considered (Fig. 1).

Meng et al. In their work, they state that the basis in determining the pathogenicity of the sequence variant of the SCN1A gene is functional changes of the alpha one subunit for the sodium channel (3). The analysis of functional changes in the sodium channel is also of irreplaceable importance in genetic counselling.

About 50 % of the identified sequence variants of the SCN1A gene are de novo. In these cases, phenotypic prediction is difficult. The so-called Truncating mutations are associated with a more severe phenotype because, due to the formation of a premature codon, they lead to the formation of a non-functional protein. In the case of missense by mutations (there is a point change in the amino acid and thus a change in the gene product), which is associated with a higher percentage with a milder phenotype, it is more difficult to predict the clinical picture without using functional studies and knowledge of the location of the pathogenic variant (3,6).

Methodology

This retrospective study included 6 patients aged 0 to 19, who underwent genetic testing. All the patients were examined using a panel of NGS epilepsy genes comprising 255 genes. For the needs of the presented work, the group included a total of 6 patients diagnosed and monitored at the Department of Pediatric Neurology, Medical Faculty of Masaryk University and University Hospital Brno, Center for Epilepsy Brno with an identified missense mutation. Informed consent for genetic testing was obtained from all the patients / parents, who underwent testing. The study was approved by the Ethical Board of the Faculty of Medicine of Masaryk University and the University Hospital Brno. Clinical

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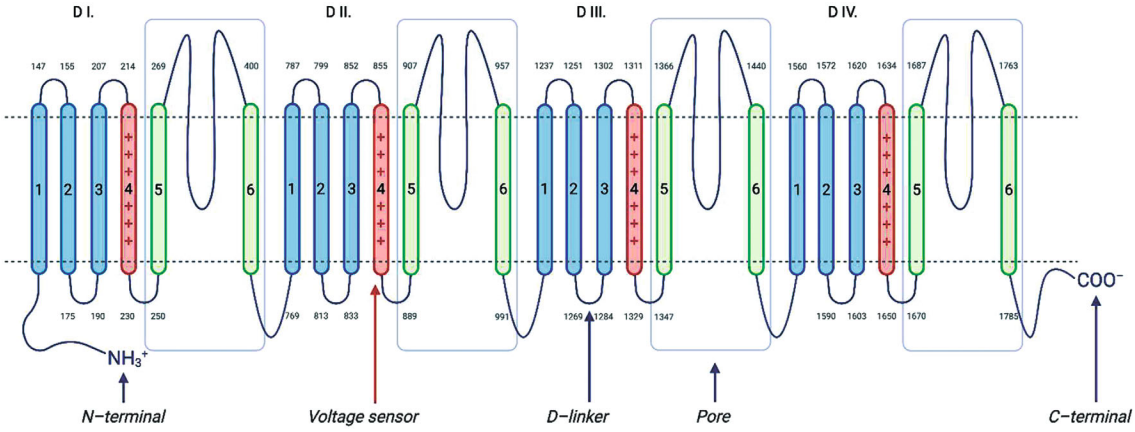

Fig. 1. Alpha 1 subunit.

characteristics of patients were retrospectively studied and the following characteristics of the studied group were selected: sex, age at onset of epilepsy, family history of epilepsy, evidence of pathogenic sequence variant in parents of the patients, provocation of seizures, individual types of epileptic paroxysms, history of epileptic states, neurological status in the patients. We further correlated the results of genetic testing with individual characteristics and clinical picture in the studied patients.

Results

The patients were born from 1986–2020, the group includes 3 women and 3 men. A more detailed description of the file together with the definition of the clinical picture is given in the Table 1.

The specific location of pathogenic sequence variants in the alpha 1 subunit for the sodium channel is shown in the scheme in Figure 2.

In 3 of our patients (50 %), we observed a milder clinical phenotype, which was mainly associated with the occurrence of recurrent febrile convulsions, isolated generalized motor paroxysms with tonic-clonic symptoms. Epileptic states were not reported in any of the patients. Objective neurological findings in these patients were normal, nor did we observe the occurrence of neurodevelopmental disorders. In 2/3 of the patients, genetic analysis showed a positive family history for an identical pathogenic sequence variant. In both patients, the carrier was the father. The parents also had a very mild course of the disease.

However, we observed the SMEI phenotype in 50 % of the patients. Epileptic states were reported in 66 % of percentages, and in all of them more types of epileptic seizures typical of this disease phenotype occured (myoclonus, generalized tonic-clonic paroxysms, focal motor seizures…). In 2/3 of the patients with a more severe phenotype, an alteration of the neurological finding was observed. No patient had a positive family history of epilepsy.

Discussion

The SCN1A gene is currently the best studied gene. Not only its structure is known, but also the function of its individual areas. More than 1200 sequence pathogenic variants of the SCN1A gene are known. This gives the epilepsy genetics its lead in mutagenicity (7).

In the group of selected patients monitored at the Department of Pediatric Neurology FM MU and FN Brno, a total of 6 patients with missense mutations were selected, because missense mutations can cause questions in predicting the patient's clinical pic-

Tab. 1. Basic data.

	Sex	Year of birth	Age at epilepsy onset (months)	Seizure provocation	Seizure type	Status epilepticus	Neurological status	Family history of epilepsy	Type of mutation	Fenotype
1.	F	1999	16	+	Febrile seizure, focal myoclonus, GTCS	0	Attention deficit, learning disability, memory deficit	0	missense	SMEI
2.	M	2015	16	+	Febrile seizure	0	normal	+ (father)	missense	GEFS+
3.	M	2017	4	+	Febrile seizure	+	normal	0	missense	SMEI
4.	M	1986	36	+	Febrile seizure GTCS	0	normal	0	missense	GEFS+
5.	F	2018	15	+	Febrile seizure	0	normal	+ (father)	missense	GEFS+
6.	M	2020	4	+	Myoclonus, GTCS	+	hypotonia central type, microcephaly, inverted mamilla	0	missense	SMEI

Red colour – SMEI phenotype, green colour - milder phenotype, SMEI – severe myoclonic epilepsy of early childhood, GEFS+ – generalized epilepsy with febrile seizures

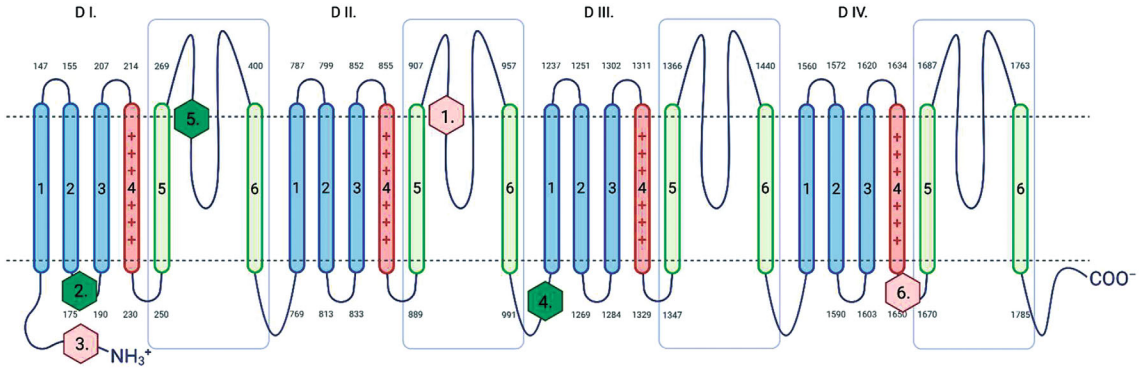

Fig. 2. Pathogenic sequence variants of Dravet patients.

ture. Based on the knowledge of the particular sequence variant, these were topped into a particular region of the alpha one subunit of the sodium channel. In the present work, the authors report the correlation of the clinical course of the disease and the localization of the pathogenic sequence variant in the SCN1A gene.

Missense mutations located in the pore region or in the region of the voltage sensor predict the so-called loss of function, or partial loss of function, i.e., severe functional impairment (LOF, p-LOF). Mutations located in the terminal region or in the region of the D-linkers, which are remote from the pore region, cause a rather milder phenotype. Missense mutations in other regions cause heterogeneous manifestations (3). However, for a more accurate analysis of a specific degree of functional damage (Tab. 2), it is necessary to perform so-called functional analyzes.

Patient sequence variant 1 was located in the pore area, which is very important for protein function and is expected to cause a more severe clinical picture in our patient accompanied by repeated generalized motor seizures, focal seizures and alterations in neurological and psychological profile, learning disability, deterioration of the patient ‘s memory functions. In the case of patients No. 2 and 4, as expected, the location of the sequence variant did not cause significant changes in the function of the protein, therefore the patients manifest themselves in a clinical picture closer to the GEFS + spectrum. The fact that the father of the child, in whom the disease also occurs with a mild course – sporadic seizures and a normal neurological profile, probably also plays an important role in patient 2.

Patient variant 6 was located in a vulnerable area, close to the pore and the voltage sensor, resulting in severe phenotypic expression. An identical pathogenic sequence variant has been described by Freilich et al. The pathogenic sequence variant was located in the short cytoplasmic linker region between transmembrane segments 4 and 5 of domain 4 of the sodium channel (10). The clinical picture of the observed patient in this work was almost identical. In both of the patients there was death in early infant resp. toddler period. The clear cause of the patient’s death in the described work was not explicitly clarified. The authors of the publication did not demonstrate by additional analysis of biological material the effect of modifying the pathogenic sequence variant in the SCN9A gene, which could potentially lead to an increase in excitability on the cell membrane. Also, no change in the copy number of the SCN1A gene was found after performing comparative hybridization (10).

Patient sequence variant 3 was located in the N-terminal region and, according to the available literature (3), it is a localization that may result in a heterogeneous clinical picture. In the case of this patient, it is an SMEI phenotype. In this case, therefore, the clinical development of the disease cannot be reliably inferred from the topification of the sequence variant in the SCN1A gene alone. To assess it, it would be appropriate to supplement the functional analysis in similar cases of sequence variant localization.

The last annotated patient in the cohort is patient 5, in whom the sequence variant was located in the pore region. Given the above information from literature, the assumption of speech was closer to the manifestations of SMEI. However, the clinical manifestation of epilepsy occured at the age of almost 3 years in the patient with normal neurological development, resp. to isolated attack of febrile convulsions. Information on identical sequence variants in the patient’s father, who did not suffer from active epilepsy and whose neurological status was normal should also be considered. The development of this patient was confirmed by the fact that even the localization of a sequence variant in the pore region did not necessarily mean a malignant phenotype, especially in the case of a missense mutation. According to the work of Meng et al. It is clear that up to 31 % of the patients with this sequence variant can be expected to have a mild course of the disease in terms of mere recurrent febrile convulsions or generalized seizures (3). The fact that almost 84 % of familial cases were accompanied by a mild phenotype (febrile convulsions or febrile convulsions + generalized paroxysms) also plays a role in the interpretation of the patient’s

Tab. 2. Definition of functional alterations of *SCN1A* gene.

Abbreviation	Function definition
LOF	loss of function
pLOF	partial loss of function
DE	decreased excitability
GOF	gain of function
IE	increased excitability
G-LOF	gain and loss of function

phenotype and localization of its sequence variant, and a negative linear relationship between phenotypic severity and familial occurrence of pathogenic sequences is described in heredity variant. Only 9.8 % of inherited mutations lead to a malignant phenotype (3). Certainly, a functional analysis of this particular sequence variant would be of great benefit in this case as well.

From the presented results of our retrospective analysis of the patients of the Medical Faculty of Masaryk University and the University Hospital in Brno, it can be seen that in all the cases the clinical phenotype of the patient can be determined with certainty only on the basis of topization of the pathogenic sequence variant. The findings correlate with world literature or. genetic databases. The most accurate is, of course, functional analysis with a precise definition of the channel function (Tab. 2).

The aim of this work was to illustrate how the studied specific sequence variants located in different parts of the Nav1.1 protein (alpha one subunit of the voltage-gated channel) cause functional changes resp. define the phenotypic image of the patient, offers a prediction of the development of the disease already in its introduction. Knowledge of the SCN1A gene today allows us to move the diagnostics even further and analyze the functional significance of individual sequence variants. Based on the results of the functional analysis of the sodium channel resp. its alteration, the phenotypic correlation is even more accurate.

It is a part of the work of a pediatric neurologist to provide comprehensive information on the prognosis of the disease, the expected response to (ASM) anti-seizure medication and also on the further neurological development of the patient. By closer functional analyses, we can better understand the variations of the clinical picture in the case of pathogenic sequence variants in the SCN1A gene, which is undoubtedly important for genetic counselling.

Next-generation sequencing methods are literally a revolution in understanding the pathophysiology of epilepsy when it comes to monogenic epilepsy. Knowledge of the pathogenic sequence variant and functional analysis of its product not only informs us about the phenotype of the patient, the prognosis of the disease, but can bring a crucial moment to the concept of precision medicine, tailor-made pharmacological treatment.

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