Open Access Article

Ann. Acad. Med. Siles. (online) 2025; 79: 384–392
eISSN 1734-025X
DOI: 10.18794/aams/211875
www.annales.sum.edu.pl

PRACA POGLĄDOWA
REVIEW

Single nucleotide variants of *MBD5* in two cases of children with mutations in the sodium channel gene *SCN1A* and *SCN9A* and a review of the literature

Warianty pojedynczego nukleotydu *MBD5* u dwojga dzieci z mutacjami w genie kanału sodowego *SCN1A* i *SCN9A* oraz przegląd literatury

Patrycja Rozwadowska-Kunecka[1,2], Małgorzata Rodak[1,3], Justyna Paprocka[4]

[1]Students' Scientific Club, Department of Pediatric Neurology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

[2]Department of Paediatrics, Diabetology and Endocrinology, University Clinical Centre, Gdańsk, Poland

[3]Centrum Zdrowia Dziecka i Rodziny im. Jana Pawła II w Sosnowcu, Poland

[4]Department of Pediatric Neurology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

ABSTRACT

Genetic factors, especially polymorphisms being a consequence of mutations in the genome, are of great importance in the etiology of drug-resistant types of epilepsy. This paper describes the cases of two patients with a very similar course of disease, both of whom were diagnosed developmental disorders, drug-resistant epilepsy and autism spectrum disorders. Both patients have *MBD5* missense mutations, while each case characterized with a different sodium channel mutation: in the case of patient 1, a 5-year-old girl, *SCN1A* gene mutation was observed, and in the case of patient 2, a 6-year-old boy, *SCN9A* mutation was recognized. No currently published articles available in medical literature have described cases with such co-occurrence of mutations so far. In both cases presented here, the recommended pharmacological treatment has not been successful, which may indicate the ineffectiveness of conventional antiseizure medications and suggest focusing on more targeted therapies.

KEYWORDS

drug resistance, epilepsy, developmental disorders, autism

Received: 24.05.2024 **Revised:** 06.10.2025 **Accepted:** 09.10.2025 **Published online:** 20.11.2025

Address for correspondence: lek. Patrycja Rozwadowska-Kunecka, Klinika Pediatrii, Diabetologii i Endokrynologii, Uniwersyteckie Centrum Kliniczne, ul. Dębinki 7, 80-952 Gdańsk, tel. +48 662 610 869, e-mail: prozwadowska@uck.gda.pl

Publisher: Medical University of Silesia, Katowice, Poland

STRESZCZENIE

Czynniki genetyczne, zwłaszcza polimorfizmy będące konsekwencją mutacji w genomie, mają duże znaczenie w etiologii lekoopornych typów padaczki. W pracy opisano przypadki dwojga pacjentów o bardzo podobnym przebiegu choroby, u których zdiagnozowano zaburzenia rozwojowe, padaczkę lekooporną i zaburzenia ze spektrum autyzmu. U obojga pacjentów stwierdzono mutacje *missense* genu *MBD5*, natomiast każdy z przypadków charakteryzował się inną mutacją kanału sodowego: w przypadku pierwszej pacjentki, 5-letniej dziewczynki, zaobserwowano mutację genu *SCN1A*, w drugim przypadku, u 6-letniego chłopca, rozpoznano mutację *SCN9A*. Dotychczas w literaturze medycznej nie opublikowano artykułów opisujących przypadki z takim współwystępowaniem mutacji. W obu przedstawionych przypadkach zalecane leczenie farmakologiczne nie przyniosło rezultatów, co może wskazywać na nieskuteczność konwencjonalnych leków przeciwpadaczkowych i sugerować skupienie się na bardziej ukierunkowanych terapiach.

SŁOWA KLUCZOWE

lekooporność, padaczka, zaburzenia rozwojowe, autyzm

INTRODUCTION

Genetic factors constitute an important element in the etiology of drug-resistant types of epilepsy. Significant advances in biomedical engineering have allowed the sequencing and identification of genes in patients suffering from epilepsy, which resulted in better clinical evaluation, as well as the development of specific personalized treatments. Among the pathogenic variants *MBD5*, *SCN1A* and *SCN9A* gene mutations can be specified [1].

The *MBD5* gene, located on chromosome 2q23.1 (#OMIM 611472) and encoding a member of the methyl-CpG binding domain (MBD) family that plays critical roles in transcriptional regulation [2], is also involved in nervous system development and behavioral regulation [3].

It contains a PWWP domain which is considered important in cell division, growth, and differentiation [2,3]. *MBD5* disorders can be associated with severe early childhood developmental encephalopathy and epilepsy [4]. The *MBD5* mutation, also referred to as mental retardation autosomal dominant 1 (MRD1) was originally described in the context of the 2q23.1 microdeletion result of which is the pseudo-Angelman syndrome [5]. Majority of phenotypes present in 2q23.1 deletion syndrom stem from haploinsufficiency of *MBD5*, while overexpression of *MBD5* leads to features of 2q23.1 duplication syndrome [3]. Nevertheless, currently used collective term describing a group of disorders associated with *MBD5* gene, *MBD5*-associated neurodevelopmental disorder (MAND), is defined as an autism spectrum disorder (ASD) characterized by intellectual disability, motor delay, severe speech impairment, and behavioral problems [3,6].

SCN1A is a gene that encodes the alpha 1 subunit of the sodium channel, which is associated with many human diseases [7], e.g. Dravet syndrome and migraine, is a member of the voltage-gated sodium channel (VGSC) family (#OMIM:182389) and has been mapped to chromosome 2q24.3 [8]. In 2000, its connection with epilepsy was also confirmed [9]. *SCN1A* is one of four sodium channel genes mainly expressed in the brain and therefore its abnormalities are related to the onset of neurodevelopmental disorders [10]. This results in a broad spectrum of phenotypes, from febrile seizures and generalized epilepsy with febrile seizures (GEFS+) to Dravet syndrome [11] characterized by polymorphic, predominantly febrile, generalized clonic or hemiclonic epileptic seizures usually manifesting in the first year of life [12]. What is more, pathogenic *SCN1A* mutations have been proven not only to occur in subjects with epileptic phenotypes, but they have also been reported in cases of patients suffering from familial hemiplegic migraines, ASDs and arthrogryposis [12]. Pathogenic *SCN1A* variants include missense and truncating variants (nonsense, frameshift and splice site), as well as intragenic microinjuries [13]. A characteristic feature of patients with mild genotypes is high frequency of missense mutations that do not result in protein truncation, whereas in severe phenotypes, missense mutations are less common [14].

SCN9A, which includes 29 exons on chromosome 2q24-q31 (#OMIM 603415) [15], is the gene encoding the alpha sodium voltage-dependent sodium channel unit Nav1.7. Nav1.7 channels placed in dorsal root ganglia and sympathetic neurons [16] play an important role in stimulating cells and gating pain transmission from the peripheral to central nervous system [17]. For that reason, mutations in the *SCN9A* gene have been identified as a cause of pain disorders [18]. Allelic conditions such as primary erythromelalgia (PE), paroxysmal extreme pain disorder (PEPD) and idiopathic small fiber neuropathy (SFN) result from the heterozygous gain-of-function *SCN9A* mutation [19], whereas loss-of-function mutations cause congeneric insensitivity to pain (CIP) and anosmia [20]. These diseases are differentiated by their phenotype, pain distribution and age of onset [15]. Currently suggested therapies for gain-of--function channelopathy include, among other ASDs, medications targeting the sodium channel, as well as

local anesthetics. Unfortunately, abovementioned therapies are inadequate, and symptoms tend to persist throughout life [15].

In the study described in this paper, two cases with *MBD5* missense mutation resulting from a single nucleotide substitution are presented. Moreover, sodium voltage-gated channels (SCN) mutation was proven to be present in both patients. Based on the available medical records, this article aims to present the developmental abnormalities that were present in both cases and which might have resulted from the diagnosed genetic mutations.

CASE REPORTS

Patient 1

The first patient, a 5.5-year-old girl, was diagnosed with epileptic encephalopathy. First epileptic seizures began to appear in the patient's 1st year of life. Since the frequency of seizures noticeably increased, the girl was admitted to the Pediatric Neurology Department at the age of 18 months. The child was from a second pregnancy, second vaginal delivery in the due date, with birth weight 3900 g; she was assigned Apgar score of 10. In the medical history of her relatives, there was a case of epilepsy in her distant family, i.e. her father's cousin. To date, psychomotor development has been described as delayed. She has not achieved milestones on time; she began to sit up at 12 months of age; at the time of the examination, she could stand up with support, and was unable to walk on her own. She did not respond to smiling, verbal contact, and did not react to her name. She did not produce any palm pointing gestures nor play; she chewed clothes and sucked her thumbs. The symptoms listed above, combined with numerous stereotypical behaviors and lack of eye contact, allowed to assume that the child have ASDs. Neurological examination showed 10th percentile head circumference (45.5 cm), decreased muscle tone, and weakly expressed tendon reflexes, especially in the lower extremities. 28 point result of the psychological examination based on the Child Development Scale placed the child under the 1st percentile, which indicated that the psychomotor development was also delayed: she pronounced only single syllables that she was not able to connect, and responded to smiles by smiling and vocalizing.

Electroencephalography (EEG) showed generalized paroxysmal activity. Despite the absence of clinical seizures, valproic acid was included into the outpatient drug treatment. Several weeks after the start of modified pharmacotherapy, due to episodes of immobility with co-occurring cyanosis around the mouth that began to appear, valproic acid was discontinued and changed to levetiracetam. After a brief period of improvement, the frequency of seizures manifested as immobility and cyanosis without convulsions increased to several times per day and they lasted up to 30 seconds. Magnetic resonance imaging (MRI) scan showed normal brain structures. On EEG examination, during hospitalization, the presence of generalized and focal paroxysmal discharges localized to the central-parieto-occipital and mid-thalamo-parietal regions. During video EEG recording, the mother reported alarming disabling incidents. At that time of brain function monitoring, no paroxysmal changes were detected.

Patient 2

A boy aged 6 years 3 months diagnosed with epilepsy and developmental delay was admitted to the Pediatric Neurology Department at the age of 2.5 years for modification of antiseizure treatment and extended diagnosis. The boy was a child from the second pregnancy, born on the due date by vaginal delivery without complications. He received Apgar score of 10/10 points with the birth weight of 4125 g. The history of psychomotor development so far was delayed. The boy started babbling at 9 months, lifted his head from 8 months, sat from 12, stood from 18, crawled from 24 months. On admission, the boy presented with autistic features, without eye and verbal contact. On neurological examination, his head circumference equalled 46.5 cm (4th percentile), also axial hypotonia and spastic paresis of the lower limbs were observed. He could walk supporting himself with his hands while his feet were internally rotated. He could stand up with support and sit up independently from a hands and knees position. The boy did not react to commands and showed no interest in toys apart from grabbing a block with his fingers or manipulating details of toys if brought close enough to his fingers. He did not speak, most frequently uttered single sounds, less often syllables, and responded to his mother's smile with a smile. Psychological examination based on the Child Developmental Scale showed delayed psychomotor development of the child. The boy scored only a few points on the applicable scale.

Patient 2 was hospitalized twice at the age of 7 and 9 months for lasting ca. 2 minutes epileptic seizures that occurred twice per day and expressed themselves in unconsciousness with increased muscle tone, and eye elevation. The EEG recording showed paroxysmal changes, which, set with seizures, allowed to diagnose epilepsy and thus pharmacotherapy with valproic acid was implemented. At the age of 11 months the boy was hospitalized again for atonic seizures which occurred 2–3 times a day. EEG and MRI were performed; epileptiform changes could be observed again in the EEG, while the brain MRI scan showed minor hypoxic-ischaemic lesions. The clinico-

-electroencephalographic correlation was confirmed. On admission at the age of 2.5 years, the patient was taking valproic acid, clobazam (since six months of age) and lamotrigine (since one month of age). The frequency of occurring atonic seizures remained on the level of 2–3 times a day. During hospitalization, the dose of lamotrigine was increased, which resulted in achieving a reduction in the number of seizures. EEG recording was abnormal, with multiple paroxysmal changes expressed in the form of numerous, generalized predominantly temporal region, 0.5–4-second paroxysmal discharges consisting of sharp waves, spikes, multi-spikes, sharp wave-slow wave complexes and occasionally spike-wave complexes of 2.4–2.9 Hz with the highest amplitude up to 580 uV in the right medial temporal region. Photic stimulation did not show any additional effect on the EEG. The electroencephalogram showed certain abnormalities and numerous generalized seizure changes; brain MRI, which was also performed again, showed apparent progression of myelination compared to the previous scan.

Molecular analysis

In both cases, the diagnosis was extended by genetic testing. The girl (patient 1) was identified with potentially pathogenic variants in two genes *MBD5* and *SCN1A*: a mutation in the *MBD5* gene with a single nucleotide substitution c.136T>G resulting in a missense mutation (p.Cys46Gly), and a mutation in the *SCN1A* gene with a single nucleotide substitution c3521C>G resulting in a missense mutation (p.Thr1174Ser). Mutations in both these genes were dominant and each occurred in a heterozygous pattern (in one allele of the gene). The boy (patient 2) was also found to have potentially pathogenic variants in two genes. In the *MBD5* gene, as in the case of patient 1, and in the *SCN9A* gene. The mutation in the *MBD5* gene involved a substitution of one c.25G>A nucleotide, resulting in a missense mutation (p.Gly9Arg). The mutation in the *SCN9A* gene involved a substitution of one c2310G>A nucleotide in exon 14 of the gene. The identified mutation was synonymous (it did not change the amino acid sequence of the encoded protein p.Leu770Leu), but due to its location in the exon/intron junction region (the splicing donor site), it may affect the correct folding of the transcript. Mutations in both genes were dominant and occurred in a heterozygous pattern.

DISCUSSION

In both patients presented, performed genetic testing showed heterozygous missense mutations of the *MBD5* gene involving a single nucleotide substitution that may potentially be of pathogenic character. While *MBD5* deletion or duplication may contribute to a genetic predisposition to ASDs, intellectual disability or epilepsy, the impact of rare single nucleotide variants (SNVs) of *MBD5* on neurodevelopmental traits has not been fully investigated [21]. Rare heterozygous *MBD5* variants were proven to be associated with the clinical heterogeneity observed in a wide range of neurodevelopmental disorders, including ASD [21].

Disruption of *MBD5* gene function has been reported to constitute a direct cause of a syndrome called MAND involving intellectual disability, severe speech impairment, epilepsy, psychiatric features of aggression and hyperactivity, and dysmorphic features including short stature and microcephaly, sleep disorders and ataxia [3,4]. MAND is a collective term describing a group of disorders associated with *MBD5* variants, which can include chromosomal deletions, duplications, disorders or intragenic single nucleotide alterations covering 2q23.1 deletion syndrome and 2q23.1 duplication syndrome. The aforementioned disorders share a common set of neurodevelopmental, cognitive, and behavioral abnormalities, but may differ in frequency and severity of symptoms [3,4]. Individuals with pathogenic variants in *MBD5* characterize with a similar but typically milder 2q23.1 deletion syndrome-like phenotype, while individuals with *MBD5*-including 2q23.1 duplication have a phenotype similar to the ones with 2q23.1 deletion. Every individual with an *MBD5* genetic anomaly is unique [3].

The clinical characteristics of presented patients with *MBD5* variants were examined retrospectively from medical records. Each case was evaluated for clinical symptoms and psychomotor development based on available sources about disruptions in the *MBD5* gene and their impact on the occurrence of developmental disorders. The clinical manifestations noticed in the case of patient 1 and patient 2 (Table I) matched those often present in MAND, including severe intellectual disability, language delay, seizures, autistic-like symptoms, stereotypical movements and motor delay [6,22]. Failure in reaching normal childhood developmental milestones in both cases contributed to

the diagnosis of neurodevelopmental disorder. The literature reports that congenital microcephaly, dysmorphic features, sleep disturbances and feeding difficulties can occur in MAND [3,6,22]. However, in none of the two patients microcephaly, craniofacial dysmorphia or skeletal changes were reported. However, it needs to be noticed that in none of the patients any mention of sleep disorders or feeding difficulties was made.

Table I. Clinical presentation (patient 1 and patient 2)

Features	Patient 1	Patient 2
Variant of *MBD5* mutation	c.136T>G	c.25G>A
Gender	F	M
Age in which examination was conducted (in years)	1.5	2.5
Developmental delay	+	+
Seizures	+	+
Speech impairment	+	+
Sleep disturbances	N	N
Behavioral problems	+	+
Feeding difficulties	N	N
Autisitc-like symptoms	+	+
Stereotypic repetitive behavior	+	+
Hypotonia	+	+
Skeletal abnormalities	N	N
Dysmorphic features	N	N
Microcephaly	–	–
Ataxic gait	+	+
Hyperphagia	N	N
Cardiovascular abnormalities	N	N

M – male; F – female; (+) feature present; (−) feature absent; N – not reported.

Both patients were identified ASD, symptoms of which included language deficits, social withdrawal and stereotypes. Currently, more than 100 genes and genomic regions have been reliably associated with autism, mostly based on studies of heterozygous, germline *de novo* mutations [23]; one of these genes is *MBD5*, which is critical for normal development. However, the biological role of methyl-CpG-binding domain 5 (*MBD5*) in neurodevelopment and ASD remains largely undefined [5]. Deletion or duplication of *MBD5* has been found to contribute to a genetic predisposition to ASD [24]. Talkowski et al. [25] proposed a mixed model of deleterious, fully penetrant *MBD5* deletions causing neurodevelopmental disorders associated with features of the 2q23.1 microdeletion syndrome and missense variants with reduced penetrance that significantly increase the risk of ASDs. In both cases presented in this paper, the autistic-like symptoms may be determined by the occurrence of SNVs resulting in a missense mutation.

In addition, the *SCN1A* mutation, which has been recognized as a constituent of ASDs as well, is also present in the case described in this article. Pathogenic variants of *SCN1A* and *SCN2A* have been identified in genetic studies of patients with ASD, which indicates its genetic etiology. Loss-of-function variants of these two genes have been recognized in post-mortem brain DNA testing of ASD patients [26]. Majority of available study publications mentions lack of verbal communication, social problems, poor peer relationships, withdrawn behavior and lack of emotional reciprocity as autistic features in patients [7]. The patient with *SCN1A* presented in this paper does not maintain eye contact, does not respond to smiles or words, and presents numerous motor stereotypies.

Myers et al. [4] analyzed the phenotypes of a global cohort of twenty-three people patients with *MBD5* deletion, duplication or point mutation and a history of epileptic seizures and found a spectrum of phenotypes associated with pathogenic *MBD5* variants that often resulted in severe developmental and epileptic encephalopathy in early childhood. Seizure incidence started at a median age of 2.9 years (range 3 days to 13 years), which suggests that the onset of epileptic seizures can occur at different stages of childhood. Developmental disorders were present in all patients: severe in 14, moderate in 8 and mild in 1. Prominent behavioral difficulties were noted in 16 patients. Childhood sleep disorders were reported in 17 patients and involved frequent nighttime awakenings. Jing et al. [27] also evaluated the clinical phenotypes and genetic features of epilepsy in 9 children with variants of the *MBD5* gene. *MBD5* gene variants include single nucleotide variations and deletions. Age of seizure onset ranged from 5 to 89 months, and multiple seizure types were observed. All patients expressed symptoms of developmental delay before the seizures started to occur: nine patients had marked language delay, and six patients had autism-like symptoms.

The heterogeneity of the *SCN1A* mutation, combined with a wide phenotypic spectrum, makes interpretation of patients' genetic tests challenging for clinicians [28]. *SCN1A* mutation can cause heterogeneous clinical phenotypes such as febrile seizures, febrile seizures plus, unclassified seizures and self-limiting focal childhood epilepsy [29]. In addition, Dravet syndrome, severe developmental and epileptic encephalopathy are caused by missense and truncation variants of the *SCN1A* protein. Patients affected by this mutation typically characterize with behavioral, motor and cognitive disorders, including depression and stroke-like episodes [30,31]. The c.3521C>G mutation in the *SCN1A* gene present in patient 1 has also been noticed in described cases of patients whose hemiplegic migraine symptoms were incidental to epilepsy symptoms [32]. Unlike other sodium channel

mutations, in which symptoms start to appear in the neonatal or early infantile period, the onset of seizures caused by the *SCN1A* mutation usually occurs in the period of infancy [28]. Patient 1, a girl with *SCN1A* mutation, diagnosed with epileptic encephalopathy, was admitted to the Pediatric Neurology Department at 18 months of age because of an increased seizure frequency. Article by Scheffer and Nabbout [7] reported that, in the case of such patients, the development usually progresses normally in the first year of life, but then rapidly slows down, usually leading to intellectual disability; patients show delayed motor skills, speech and language, as well as social skills [8]. Patient 1, presented in this article, did not reach normal milestones for her age and presented delayed psychomotor development. Problems appeared also in the area of speech and word formation.

Mutation in the *SCN9A* gene is expressed in different genotype-phenotype forms. Some patients with *SCN9A* mutation meet clinical criteria for erythromelalgia, which include the presence of severe, temperature-related pain and erythema in the feet, hands and ears (aggravated by heat and relieved by cold) [33]. The described boy with the *SCN9A* mutation does not present the abovementioned characteristics, which may be related to the young age of the patient, as cases of onset of symptoms at the age of 6 years are described in the literature [34]. In addition to erythromelalgia, patients with the *SCN9A* mutation may also show psychomotor retardation along with pain attacks that are resistant to analgesic treatment. Severe pain attacks contribute to reduced social interactions and may lead to self--destructive behaviors (such as self-injury) [15]. The *SCN9A* mutation also causes PEPD, which characterizes with present from birth recurrent seizures with closed or staring eyes, limb stiffness, cessation of crying in the neonatal period, redness of the skin and trunk along with cyanosis in the face and mouth area. While the process of intellectual development of patients with PEPD progresses normally, their motor development is delayed [35]. Another extremely rare disorder caused by loss-of--function mutations in the *SCN9A* gene is CIP. Patients suffering from it are incapable of feeling pain and show no response to injuries [36]. The *SCN9A* mutation is also suspected of being one of the possible causes of epilepsy, but this correlation is still being discussed among the research communities [37]. Albaradie et al. [38] described a patient with an identified *SCN9A* mutation who complained of myoclonic seizures which frequency of occurrence ranging from 10 to 15 times a day. Furthermore, the patient was diagnosed with global developmental delay: even though the patient was walking, he experienced frequent falls due to myoclonic jerks, and tremor impaired his normal daily activities. In addition, the patient had significant difficulties in school. Among the cases of patients with the *SCN9A* mutation and epilepsy described in literature, there is an interesting study of twin sisters. Liu et al. [39] reported on two 10-year-old siblings with a normal birth and developmental history. Their first clonic seizures started to appear at the age of 7 as an incident of a nocturnal clonic seizure involving movements of the right upper limb and an oropharyngeal region, while a generalized tonic-clonic seizure appeared a few months later. The subsequent diagnosis indicated Roland's epilepsy. Similarly, patient 2 reported in this paper was diagnosed with epilepsy and developmental delay, and did not reach normal developmental milestones for his age. A severe reduction in muscle tone, which occurred when he was 2.5 years old, was followed with his significant problems with walking. The patient also showed features of autism, such as short eye contact, difficulty in connecting and lack of speaking, replaced with pronouncing only single sounds. The boy experienced seizures in form of unconsciousness with increased muscle tension and supraduction.

In the article assessing the phenotypic spectrum of seizure disorders in MAND Myers et al. [4] closely examined EEG graphs of patients were abnormal in 17/21 cases, and usually showed slow generalized spike wave complexes and background slowing. An important aspect for this paper is that electroencephalograms of both patients described here presented paroxysmal discharges. In a study described by Jing et al. [27] comparing nine patients with different *MBD5* mutation variants, five patients had slow background activity in the EEG. Interictal EEG showed abnormal discharges in nine patients. It is worth noting that brain MRI was normal in all patients.

EEG in patients with *SCN1A* mutation is normal at the onset of the disease [7]. In the research performed by Kong et al. [28] on a group of patients with these mutations, almost half of the interictal EEG recordings were normal, and more than a half of the recordings showed focal epileptiform discharges. In comparison Ma et al. [8] found epileptiform discharges also in the interictal phase in most of the studied cases. EEG examination of patient 1 presented abnormalities with generalized and focal epileptic discharges.

The patient whose mutation in *SCN9A* caused PEPD on EEG did not present interictal or symptomatic abnormalities [35]. In comparison, the patient with this mutation causing epilepsy on EEG recorded generalized epileptiform discharges and multiple polymorphic seizures [38]. In the abovementioned twin sisters with Roland's epilepsy, the EEG graph showed prominent interictal high-voltage spike and spike-and-slow waves in the bilateral medial temporal

regions, which were exacerbated during sleep [39]. In the reported patient 2, the EEG showed an abnormal recording with multiple paroxysmal discharges.

MAND is usually associated with normal brain neuroimaging or thin corpus callosum with mild hypomyelination in rare cases. There are rare reports of focal cerebral malformations with pathogenic *MBD5* variants but these have been associated with relatively large heterozygous deletions involving loss of multiple genes other than *MBD5* [40,41]. In hereby paper, MRI of both patients showed normal images of brain structures.

Brain MRI examinations in patients with *SCN1A* mutations usually show no abnormalities [7]. Similarly, MRI imaging of brain structures of patient 1 also showed no brain structural changes.

In a paper by Meijer et al. [15] describing an atypical case of SCN9A mutation of a patient with paroxysmal pain attacks, with sweating and erythema of her lower limbs and hands with a slight asymmetry of the anterior ventricular horns on computed tomography scans. In contrast, the patient with PEPD and the patient with congenital insensitivity to pain described in the article by Hua et al. [35], Sun et al. [36] showed no abnormalities on head MRI. In a patient observed in the research by Albaradie et al. [38] diagnosed with epilepsy and a mutation in SCN9A, MRI revealed mild generalized atrophy. In twin sisters from Liu et al. [39] study head MRI was normal. Similarly, in the case of the presented patient with SCN9A mutation, the head MRI showed a brain image within normal limits with apparent progression of myelination compared to the previous MRI.

Epilepsy associated with variants of the *MBD5* gene is usually refractory to treatment [4,27]. Anticonvulsant drugs such as valproate, clonazepam, zonisamide and clobazam usually used in treatment prove to be effective in reducing the incidence of seizures [3]. In a study evaluating the phenotypic spectrum of seizure disorders in MAND ten of the patients had previously been diagnosed drug-resistant epilepsy [4]. Although none of the substances drug was recognized as clearly superior, valproate showed the most consistent beneficial effect (12/14 cases), while carbamazepine exacerbated seizures in one patient. There is currently no cure for MAND, and treatment is based on managing the symptoms of the disorder. Treatment typically involves a combination of therapies, including behavioral and educational interventions, speech and language therapy, as well as physical and occupational therapy [3,42]. Medications may be used to manage specific symptoms, such as epileptic seizures or sleep disturbances. In terms of targeted therapies for the specific *MBD5* mutation, there are currently no approved treatments. However, the ongoing research will allow to develop new therapies and understand the underlying biology of MAND better. Gene therapy and other precision medicine approaches may hold promise for a successful treatment of genetic disorders like MAND in the future [42].

Epilepsy caused by *SCN1A* mutation characterizes with drug-resistance and requires multidirectional therapy [7]. The literature indicates that the most commonly used drugs are sodium valproate and levetiracetam, stiripentol, fenfluramine, cannabidiol, which are effective in reducing seizure frequency [43]. Antiepileptic drugs such as sodium channel blockers may exacerbate the seizures [44]. Most *SCN1A* mutations tend to be of loss-of-function type. Advances in genetic research and more detailed biophysical analysis allowed to discover new mutation variants resulting in an increase in channel function or decrease, as well as a mixture of loss- and gain-of--function. This heterogeneity of mutations is the reason why a treatment effective for one patient, may exacerbate symptoms in another [45]. It is important to individualize patient therapy and gain a better molecular understanding in the aspect of the range of mutation variants.

The individually recommended pharmacotherapy would, thus, depend on the phenotypic form associated with the mutation in the *SCN9A* gene. In erythromelalgia, sodium channel blockers such as mexiletine, lidocaine and carbamazepine are used, however, the scope of information regarding their efficacy is limited [33]. In patients with pain attacks, a partial therapeutic response was achieved with carbamazepine, to which mexiletine was added [15]. A patient with PEPD, treatment with carbamazepine allowed to achieve an improvement in motor skills and reduction in seizure frequency [35]. Another patient with recognized *SCN9A* mutation and epilepsy was treated with sodium valproate, which initially ceased the seizures, but after several months the patient's condition deteriorated and sodium valproate was changed to levetiracetam, which resulted in seizure reduction to some extent [38]. In the case of twins, seizure control was achieved after

administration of oxcarbazepine; however, their pharmacological treatment included levetiracetam as well. After noticing a recurrence of seizures clonazepam was introduced, which resulted in patients remaining seizure-free [39]. The patient described in the paper was treated with valproic acid, clonazepam and lamotrigine. Adjustment of lamotrigine dose reduced the number of seizures, but the boy is still not seizure-free.

CONCLUSIONS

In the study we present two cases of patients with a very similar course of disease, whose common feature is the presence of a missense *MBD5* mutation resulting from a single nucleotide substitution. On this basis, we can speculate that the presence of this mutation results in developmental disorders, epilepsy and ASDs, in each of the presented patients. Nevertheless, patients differ in the co-occurrence of other sodium channel mutations *SCN1A* and *SCN9A*, respectively. To date, no patients with such co-occurrence of mutations have been described. It is not known whether the co-occurrence of both mutations deteriorates the course of the disease in children, but it is certain that these mutations are also linked to developmental disorders, including epilepsy and ASD. Diagnosis of these mutations is extremely difficult, as evidenced by the small number of cases described to date due patients presenting very different phenotypes. Furthermore, the observed differences in genotype-phenotype characters pose another difficulty in terms of patients' comparison and make it impossible to establish a definite treatment. The presented patients suffer from drug-resistant epilepsies, which may indicate that conventional antiepileptic treatment in patients with genetic background of epilepsy is ineffective, and therefore it is worth to focus more on targeted treatments. Also, further follow-up of the patients is required for future delineation of the new phenotype of co-occurrence of some pathogenic variants.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Informed consent statement

Written informed consent was obtained from the patient's parents.

Ethical approval

Ethical review and approval was waived for this study due to the description of individual cases with the informed consent of the participant.

Funding

This research received no external funding.

Authors' contribution

Study design – P. Rozwadowska-Kunecka, M. Rodak, J. Paprocka
Data collection – P. Rozwadowska-Kunecka, M. Rodak
Manuscript preparation – P. Rozwadowska-Kunecka, M. Rodak, J. Paprocka
Literature research – P. Rozwadowska-Kunecka, M. Rodak
Final approval of the version to be published – P. Rozwadowska-Kunecka, J. Paprocka

REFERENCES

1. Wang Y, Du X, Bin R, Yu S, Xia Z, Zheng G, et al. Genetic Variants Identified from Epilepsy of Unknown Etiology in Chinese Children by Targeted Exome Sequencing. Sci Rep. 2017;7:40319. doi: 10.1038/srep40319.

2. National Center for Biotechnology Information (NCBI). National Library of Medicine (US), National Center for Biotechnology Information, 1988 [online] https://www.ncbi.nlm.nih.gov/gene/55777#reference-sequences [accessed on 29 August 2023].

3. Mullegama SV, Elsea SH. Clinical and Molecular Aspects of MBD5--Associated Neurodevelopmental Disorder (MAND). Eur J Hum Genet. 2016;24(9):1235–1243. doi: 10.1038/ejhg.2016.35.

4. Myers KA, Marini C, Carvill GL, McTague A, Panetta J, Stutterd C, et al. Phenotypic Spectrum of Seizure Disorders in MBD5-Associated Neurodevelopmental Disorder. Neurol Genet. 2021;7(2):e579. doi: 10.1212/NXG.0000000000000579.

5. Marco EJ, Aitken AB, Nair VP, da Gente G, Gerdes MR, Bologlu L, et al. Burden of de novo mutations and inherited rare single nucleotide variants in children with sensory processing dysfunction. BMC Med Genomics. 2018;11(1):50. doi: 10.1186/s12920-018-0362-x.

6. Mullegama SV, Klein SD, Williams SR, Innis JW, Probst FJ, Haldeman--Englert C, et al. Transcriptome analysis of MBD5-associated neurodevelopmental disorder (MAND) neural progenitor cells reveals dysregulation of autism-associated genes. Sci Rep. 2021;11(1):11295. doi: 10.1038/s41598-021-90798-z.

7. Scheffer IE, Nabbout R. SCN1A-related phenotypes: Epilepsy and beyond. Epilepsia. 2019;60 Suppl 3:S17–S24. doi: 10.1111/epi.16386.

8. Ma R, Duan Y, Zhang L, Qi X, Zhang L, Pan S, et al. SCN1A-Related Epilepsy: Novel Mutations and Rare Phenotypes. Front Mol Neurosci. 2022;15:826183. doi: 10.3389/fnmol.2022.826183.

9. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet. 2000;24(4):343–345. doi: 10.1038/74159.

10. Brunklaus A, Brünger T, Feng T, Fons C, Lehikoinen A, Panagiotakaki E, et al. The gain of function SCN1A disorder spectrum: novel epilepsy phenotypes and therapeutic implications. Brain. 2022;145(11):3816–3831. doi: 10.1093/brain/awac210.
11. Clatot J, Parthasarathy S, Cohen S, McKee JL, Massey S, Somarowthu A, et al. SCN1A gain-of-function mutation causing an early onset epileptic encephalopathy. Epilepsia. 2023;64(5):1318–1330. doi: 10.1111/epi.17444.
12. Moretti R, Arnaud L, Bouteiller D, Trouillard O, Moreau P, Buratti J, et al. SCN1A-related epilepsy with recessive inheritance: Two further families. Eur J Paediatr Neurol. 2021;33:121–124. doi: 10.1016/j.ejpn.2021.05.018.
13. Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype-phenotype associations in SCN1A-related epilepsies. Neurology. 2011;76(7):594–600. doi: 10.1212/WNL.0b013e31820c309b.
14. Meng H, Xu HQ, Yu L, Lin GW, He N, Su T, et al. The SCN1A mutation database: updating information and analysis of the relationships among genotype, functional alteration, and phenotype. Hum Mutat. 2015;36(6):573–580. doi: 10.1002/humu.22782.
15. Meijer IA, Vanasse M, Nizard S, Robitaille Y, Rossignol E. An atypical case of SCN9A mutation presenting with global motor delay and a severe pain disorder. Muscle Nerve. 2014;49(1):134–138. doi: 10.1002/mus.23968.
16. Black JA, Waxman SG. Sodium channel expression: a dynamic process in neurons and non-neuronal cells. Dev Neurosci. 1996;18(3):139–152. doi: 10.1159/000111403.
17. Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium channels in normal and pathological pain. Annu Rev Neurosci. 2010;33:325–347. doi: 10.1146/annurev-neuro-060909-153234.
18. Waxman SG. Channelopathic pain: a growing but still small list of model disorders. Neuron. 2010;66(5):622–624. doi: 10.1016/j.neuron.2010.05.029.
19. Faber CG, Hoeijmakers JG, Ahn HS, Cheng X, Han C, Choi JS, et al. Gain of function Nav1.7 mutations in idiopathic small fiber neuropathy. Ann Neurol. 2012;71(1):26–39. doi: 10.1002/ana.22485.
20. Cox JJ, Sheynin J, Shorer Z, Reimann F, Nicholas AK, Zubovic L, et al. Congenital insensitivity to pain: novel SCN9A missense and in-frame deletion mutations. Hum Mutat. 2010;31(9):E1670–E1686. doi: 10.1002/humu.21325.
21. Ishizuka K, Kimura H, Yoshimi A, Banno M, Kushima I, Uno Y, et al. Investigation of single-nucleotide variants in MBD5 associated with autism spectrum disorders and schizophrenia phenotypes. Nagoya J Med Sci. 2016;78(4):465–474. doi: 10.18999/nagjms.78.4.465.
22. Williams SR, Mullegama SV, Rosenfeld JA, Dagli AI, Hatchwell E, Allen WP, et al. Haploinsufficiency of MBD5 associated with a syndrome involving microcephaly, intellectual disabilities, severe speech impairment, and seizures. Eur J Hum Genet. 2010;18(4):436–441. doi: 10.1038/ejhg.2009.199.
23. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An JY, et al. Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. Cell. 2020;180(3):568–584.e23. doi: 10.1016/j.cell.2019.12.036.
24. Mullegama SV, Rosenfeld JA, Orellana C, van Bon BWM, Halbach S, Repnikova E, et al. Reciprocal deletion and duplication at 2q23.1 indicates a role for MBD5 in autism spectrum disorder. Eur J Hum Genet. 2014;22(1):57–63. doi: 10.1038/ejhg.2013.67.
25. Talkowski ME, Mullegama SV, Rosenfeld JA, van Bon BWM, Shen Y, Repnikova EA, et al. Assessment of 2q23.1 microdeletion syndrome implicates MBD5 as a single causal locus of intellectual disability, epilepsy, and autism spectrum disorder. Am J Hum Genet. 2011;89(4):551–563. doi: 10.1016/j.ajhg.2011.09.011.
26. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat Genet. 2011;43(6):585—589. doi: 10.1038/ng.835.
27. Jing XW, Cheng MM, Niu XY, Yang Y, Yang XL, Yang ZX, et al. Clinical phenotypes and genetic features of epilepsy children with MBD5 gene variants. [Article in Chinese]. Zhonghua Er Ke Za Zhi. 2022;60(4):345––349. doi: 10.3760/cma.j.cn112140-20211015-00874.
28. Kong Y, Yan K, Hu L, Wang M, Dong X, Lu Y, et al. Association between SCN1A and SCN2A mutations and clinical/EEG features in Chinese patients from epilepsy or severe seizures. Clin Chim Acta. 2018;483:14–19. doi: 10.1016/j.cca.2018.03.027.
29. Kivity S, Oliver KL, Afawi Z, Damiano JA, Arsov T, Bahlo M, et al. SCN1A clinical spectrum includes the self-limited focal epilepsies of childhood. Epilepsy Res. 2017;131:9–14. doi: 10.1016/j.eplepsyres.2017.01.012.
30. Brunklaus A, Du J, Steckler F, Ghanty II, Johannesen KM, Fenger CD, et al. Biological concepts in human sodium channel epilepsies and their relevance in clinical practice. Epilepsia. 2020;61(3):387–399. doi: 10.1111/epi.16438.
31. Fry AE, Rees E, Thompson R, Mantripragada K, Blake P, Jones G, et al. Pathogenic copy number variants and SCN1A mutations in patients with intellectual disability and childhood-onset epilepsy. BMC Med Genet. 2016;17(1):34. doi: 10.1186/s12881-016-0294-2.
32. Hasirci Bayir BR, Tutkavul K, Eser M, Baykan B. Epilepsy in patients with familial hemiplegic migraine. Seizure. 2021;88:87–94. doi: 10.1016/j.seizure.2021.03.028.
33. Arthur L, Keen K, Verriotis M, Peters J, Kelly A, Howard RF, et al. Pediatric Erythromelalgia and SCN9A Mutations: Systematic Review and Single-Center Case Series. J Pediatr. 2019;206:217–224.e9. doi: 10.1016/j.jpeds.2018.10.024.
34. Feng S, He Z, Que L, Luo X, Liang L, Li D, et al. Primary erythromelalgia mainly manifested by hypertensive crisis: A case report and literature review. Front Pediatr. 2022;10:796149. doi: 10.3389/fped.2022.796149.
35. Hua Y, Cui D, Han L, Xu L, Mao S, Yang C, et al. A novel SCN9A gene variant identified in a Chinese girl with paroxysmal extreme pain disorder (PEPD): a rare case report. BMC Med Genomics. 2022;15(1):159. doi: 10.1186/s12920-022-01302-z.
36. Sun J, Li L, Yang L, Duan G, Ma T, Li N, et al. Novel *SCN9A* missense mutations contribute to congenital insensitivity to pain: Unexpected correlation between electrophysiological characterization and clinical phenotype. Mol Pain. 2020;16:1744806920923881. doi: 10.1177/1744806920923881.
37. Brunklaus A. No evidence that SCN9A variants are associated with epilepsy. Seizure. 2021;91:172–173. doi: 10.1016/j.seizure.2021.05.026.
38. Albaradie R, Baig DN, Bashir S. Sodium voltage-gated channel alpha subunit 9 mutation in epilepsy. Eur Rev Med Pharmacol Sci. 2021;25(24):7873–7877. doi: 10.26355/eurrev_202112_27635.
39. Liu Z, Ye X, Qiao P, Luo W, Wu Y, He Y, et al. G327E mutation in SCN9A gene causes idiopathic focal epilepsy with Rolandic spikes: a case report of twin sisters. Neurol Sci. 2019;40(7):1457–1460. doi: 10.1007/s10072-019-03752-3.
40. van Bon BWM, Koolen DA, Brueton L, McMullan D, Lichtenbelt KD, Adès LC, et al. The 2q23.1 microdeletion syndrome: clinical and behavioural phenotype. Eur J Hum Genet. 2010;18(2):163–170. doi: 10.1038/ejhg.2009.152.
41. Jaillard S, Dubourg C, Gérard-Blanluet M, Delahaye A, Pasquier L, Dupont C, et al. 2q23.1 microdeletion identified by array comparative genomic hybridisation: an emerging phenotype with Angelman-like features? J Med Genet. 2009;46(12):847–855. doi: 10.1136/jmg.2008.058156.
42. Mullegama SV, Mendoza-Londono R, Elsea SH. *MBD5* Haploinsufficiency. In: Adam MP, Mirzaa GM, Pagon RA, et al. [eds.]. GeneReviews. University of Washington, Seattle; 1993-2023 [online] https://www.ncbi.nlm.nih.gov/books/NBK390803/ Updated 2022 Apr 28 [accessed on 15 April 2023].
43. Striano P, Coppola A, Pezzella M, Ciampa C, Specchio N, Ragona F, et al. An open-label trial of levetiracetam in severe myoclonic epilepsy of infancy. Neurology. 2007;69(3):250–254. doi: 10.1212/01.wnl.0000265222.24102.db.
44. Sahli M, Zrhidri A, Elaloui SC, Smaili W, Lyahyai J, Oudghiri FZ, et al. Clinical exome sequencing identifies two novel mutations of the SCN1A and SCN2A genes in Moroccan patients with epilepsy: a case series. J Med Case Rep. 2019;13(1):266. doi: 10.1186/s13256-019-2203-8.
45. Jones LB, Peters CH, Rosch RE, Owers M, Hughes E, Pal DK, et al. The L1624Q Variant in *SCN1A* Causes Familial Epilepsy Through a Mixed Gain and Loss of Channel Function. Front Pharmacol. 2021;12:788192. doi: 10.3389/fphar.2021.788192.