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From focal epilepsy to Dravet syndrome – Heterogeneity of the phenotype due to SCN1A mutations of the p.Arg1596 amino acid residue in the Nav1.1 subunit

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ABSTRACT

Objective: The aim of this study was to analyze the intra-/interfamilial phenotypic heterogeneity due to variants at the highly evolutionary conservative p.Arg1596 residue in the Nav1.1 subunit.

Materials/participants: Among patients referred for analysis of the *SCN1A* gene one recurrent, heritable mutation was found in families enrolled into the study. Probands from those families even clinically diagnosed with atypical Dravet syndrome (DS), generalized epilepsy with febrile seizures plus (GEFS+), and focal epilepsy, had heterozygous p.Arg1596 His/Cys missense substitutions, c.4787G > T and c.4786C > T in the *SCN1A* gene.

Method: Full clinical evaluation, including cognitive development, neurological examination, EEGs, MRI was performed in probands and affected family members in developmental age. The whole *SCN1A* gene sequencing was performed for all probands. The exon 25, where the identified missense substitutions are localized, was directly analyzed for the other family members.

Results: Mutation of the *SCN1A* p.1596Arg was identified in three families, in one case substitution p.Arg1596Cys and in two cases p.Arg1596His. Both mutations were previously described as pathogenic and causative for DS, GEFS+ and focal epilepsy. Spectrum of phenotypes among presented families with p.Arg1596 mutations shows heterogeneity ranged from asymptomatic cases, through FS and FS+ to GEFS+/Panayiotopoulos syndrome and epilepsies with and without febrile seizures, and epileptic encephalopathy such as DS. Phenotypes differ among patients displaying both focal and generalized epilepsies. Some patients demonstrated additionally Asperger syndrome and ataxia.

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Conclusion: Clinical picture heterogeneity of the patients carrying mutation of the same residue indicates the involvement of the other factors influencing the *SCN1A* gene mutations' penetrance.

1. Introduction

Mutations in the gene *SCN1A* [MIM 182389] coding for the α1 subunit of the neuronal sodium channel (Nav1.1) have been associated with various types of epilepsy. Clinical spectrum of *SCN1A* mutations ranges from febrile seizures and quite benign febrile seizures plus (FS or FS+, MIM 604403), genetic epilepsy with febrile seizures plus (GEFS+, MIM 604403) to severe epilepsy syndromes such as Dravet syndrome (DS, MIM 607208) and intractable childhood epilepsy with generalized tonic–clonic seizures (ICE-GTC) [1]. *SCN1A* mutations have been also found in single patients with other epilepsy syndromes (*e.g.* West syndrome [2], Lennox–Gastaut syndrome [3], Rasmussen encephalitis [4] and Panayiotopoulos syndrome [5]) and in rare cases of Familial Hemiplegic Migraine (FHM, MIM 6096345) [6] and Familial Autism [7].

So far more than 1200 different mutations in the *SCN1A* gene have been identified [according to SCN1A Mutation Database, http://www.gzneurosci.com/scn1adatabase/]. In the most severe cases, like Dravet syndrome, mutations mainly arise *de novo* (90%), in less severe phenotypes like GEFS+ and in rare cases of DS are hereditary [1]. Recurrent mutations in the *SCN1A* gene are rather rare. Because of that, identification the groups of patients, especially familial cases, with the same mutations enable the analysis of the possible phenotypic heterogeneity due to identical/similar changes. Some components of this variability are likely to be related to genetic factors [8,9]. The heterogeneity in clinical course, especially observed intrafamilial may help in identification of the SCN1A-related disorders course modifiers.

Hereby, we present three unrelated families with confirmed missense mutations – p.Arg1596His and p.Arg1596Cys in the *SCN1A* gene, in order to analyze epilepsy phenotype spectrum in affected individuals due to different changes at this amino acid residue.

2. Materials and methods

2.1. Subjects

The *SCN1A* gene analysis was performed in the group of patients clinically diagnosed with DS/DS-Borderline or GEFS+ syndrome. Three unrelated families with p.Arg1596 His/Cys missense substitutions were enrolled into this study. Mutations were identified in probands clinically diagnosed as DS, GEFS+ and focal epilepsy.

Phenotypes of epilepsies were assessed according to International League Against Epilepsy (ILAE) classification system [10,11].

When hereditary character of the identified mutations was confirmed, the detailed clinical history was collected through interviewing patients' parents and other relatives. Probands and the other available affected subjects underwent full neurological examinations performed by neuropediatricians. Interictal electroencephalograms (EEGs) and magnetic resonance imaging (MRI) were performed in probands and patients when possible. Clinical course of the remaining subjects was obtained by reviewing available medical records to extract information about the seizure onset and history, EEG records, neurological examination and brain imaging findings as well as antiepileptic treatment.

Blood samples were collected from 17 affected individuals and their 8 asymptomatic relatives. All participants (parents and adult patients) signed the informed consent form.

The Ethics Committee of the Institute of Mother and Child approved the study protocol.

2.2. Subjects' clinical assessment

Pedigrees of all families are presented in Fig. 1; the main clinical data are summarized in Table 1

2.2.1. Family 1 (Fam1RC)

Four affected and one asymptomatic individuals being p. Arg1596Cys mutation carriers were identified in this family. The proband (III-2) was 6-year-old boy finally diagnosed with atypical Dravet syndrome. The patient had an uneventful prenatal history and normal psychomotor development in the first 3 years of life. He developed generalized tonic–clonic seizures (GTCS) associated with high fever at 14 months of age. Seizures recurrence appeared after two years. Since then he had experienced different types of seizures including absences, myoclonic, GTCS, focal unilateral, alternating predominantly on the right and vegetative, frequently in clusters, associated or not with fever. The status epilepticus was also observed. The patient was treated with VPA, and then for short time oxcarbazepine (OXCB), and lamotrigine (LTG) was added, without clinical course improvement. The seizure remission was achieved after introduction of levetiracetam (LEV) added to VPA at the age of 5 years. The improvement of his cognitive functions was observed (change of IQ from 120 at 4.5 years of age to 140 pts at 6 years, by Wechsler test) during remission but the Asperger Syndrome and dyspraxia were developed.

Both proband's father (II-1) and his brother (II-4) had normal intelligence and were graduated from university. The first one had suffered from epilepsy with GTCS since the age of 13 years. He was treated with CBZ and nevertheless he has achieved remission at the age of 39 years. The second one (II-4), had his first GTCS at the age of 4 years during physical effort

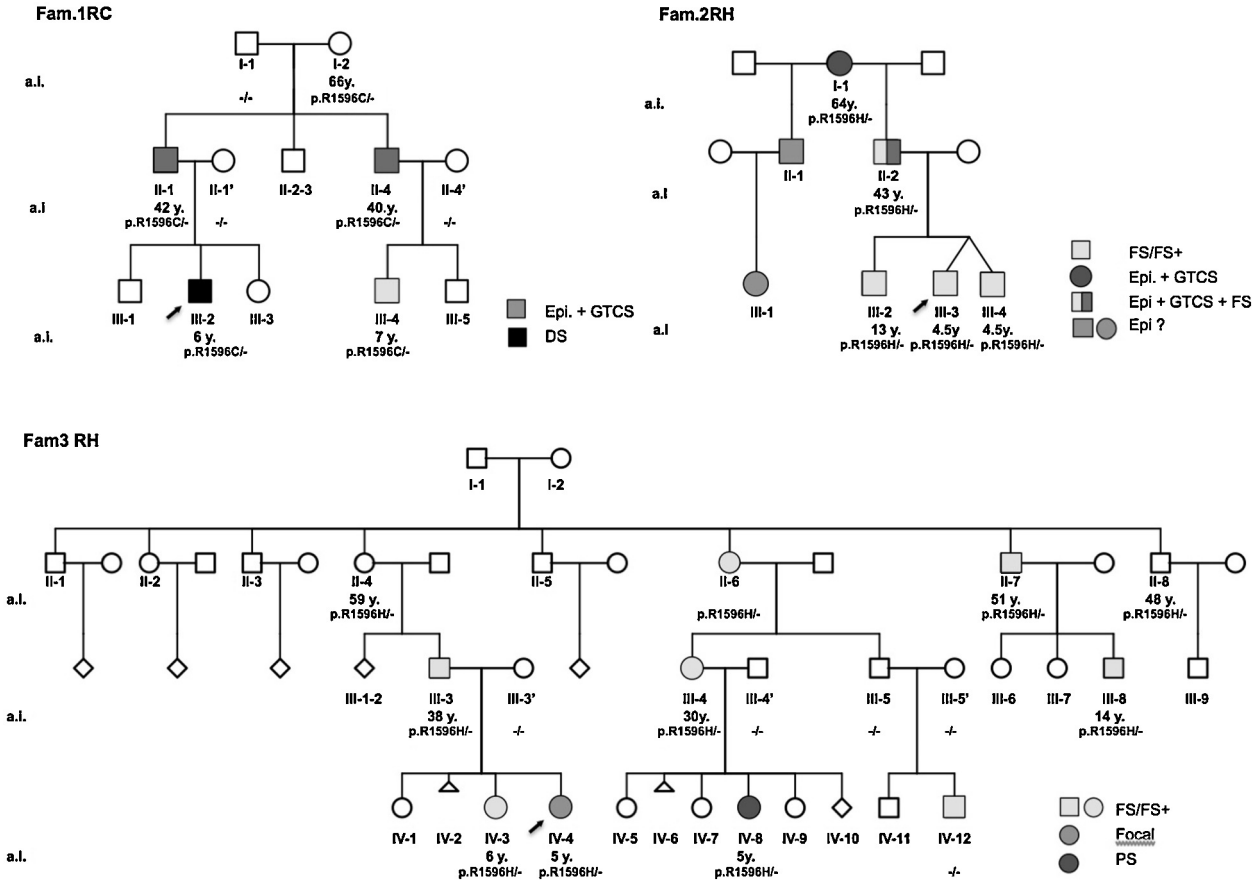

Fig. 1 – Pedigree diagrams of the families with the p.Arg1596 residue substitutions p.Arg1596Cys (FAM.1RC) and p.Arg1596His (Fam.2RH, Fam.3RH). Squares represent males and circles represent females. Arrows indicates probands. Phenotypes are described for each diagram. In mutation description the one letter amino acid code was used; R = Arg, H = His, C = Cys, description of the genotypes according to HGVS v.2 nomecnclature should be as follows: p.[Arg1596Cys];[=], p.[Arg1596His];[=], [=];[=] (HGVS v.2 nomenclature Dunnen JT, Antonarakis SE 2000 [31]). *Abbreviations*: a.i. – age at investigation; epilepsy phenotypes: Epi. – epilepsy; GTCS – generalized tonic-clonic seizures; Focal – partial seizures; FS – febrile seizures; FS+ – febrile seizures plus; DS – Dravet syndrome; PS – Panayiotopulos syndrome.

(hyperthermia?). He was treated with CBZ replaced then by OXCB without success. He achieved seizures remission at the age of 39 years, when the alternative VPA monotherapy was introduced. Proband's cousin (III-4) with normal cognitive development was diagnosed with FS+ and treated with VPA. Slight ataxia and clumsiness were found in his neurological assessment. Proband's paternal grandmother (I-1) was asymptomatic mutation's carrier of normal intelligence.

2.2.2. *Family 2 (Fam.2 RH)*

In this family 7 affected subjects, with five being a carrier of p. Arg1596His mutation, over three generations were identified. The proband (III-3) diagnosed with FS+ was 4.5-year-old boy with normal psychomotor and intellectual development. His first GTCS was associated with high fever at 12 months of life. Over the next 2 years he experienced a few, both febrile and afebrile GTCS. The VPA treatment introduced after his first afebrile seizure was successful. The proband's twin brother (III-2) displayed similar course of disease.

Proband's older brother (III-2), developed one simple FS at the age of 12 months. His cognitive development was normal, but he suffered from migraine without aura and motion sickness. Proband's father (II-2) was 34 years old, with normal intelligence. He developed epilepsy with GTCS in the first year of life (no information upon relation with fever). He was treated with bitherapy of VPA and carbamazepine (CBZ) without remission of seizures. Proband's paternal grandmother (I-1) had suffered from epilepsy with GTCS since 12 years. She had achieved full remission of seizures at the age of 50 years while being treated with CBZ. Patient II-1 (I-1 son) was 43 years old and had normal intelligence. He experienced one FS during the first year of life. He has developed a few GTCS since the age of 41. He received no treatment, as he was addicted to alcohol. Patient III-1 was 21-year-old daughter of patient II-1. She was born with severe asphyxia and was mentally retarded. She experienced a few FS during infection in the first year of life and then developed refractory epilepsy with GTCS, but precise data concerning both epilepsy phenotype and AEDs

Table 1 – Clinical characterization of the p.Arg1596Cys and p.Arg1596His mutation carriers in presented families.

	p.Arg1596Cys Fam1RC					p.Arg1596His Fam2RH				
ID	I-2	II-1	II-4	III-2 proband	III-4	I-1	II-2	III-2	III-3 proband	III-4
Mutation	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–
Gender	F	M	M	M	M	F	M	M	M	M
Age at investigation	66 y	42 y	40 y	6 y	7 y	64 y	34 y	12.5 y	4.5 y	4.5 y
Age at seizure onset/remission	–	13 y/39 y	4 y/39 y	14 m/4 y	3 y/6 y	12 y/50 y	1 y/No	12 m/12 m	12 m/3 y	13 m/No
First seizures type	–	Afebrile	Physical effort hyperthermia (?)	Febrile GTCS	Febrile GTCS	Afebrile	Febrile GTCS	Febrile GTCS	Febrile GTCS	Febrile GTCS
FS	–	No	No	Yes	Yes	ND	Yes	Yes	Yes	Yes
Status Epilepticus	–	No	No	Yes, clusters of seizures	Yes	No	No	No	No	No
Subsequent seizures types	–	GTCS	GTCS	Myoclonic, GTCS, focal unilateral, alternating predominantly on the right, vegetative	GTCS	GTCS	GTCS	GTCS	GTCS	GTCS
Interictal EEG	ND	ND	ND	Focal, lateralized and generalized spikes/ multispikes, spikes-waves predominantly on the left site	Normal	ND	ND	Normal	Generalized spike-wave complexes	Generalized spike-wave complexes
Neuroimaging	ND	ND	ND	Normal	ND	ND	ND	Normal	ND	ND
Clinical examination	Normal	Normal	Normal	dyspraxia	Ataxia clumsiness	Normal	Normal	Normal, migraine	Normal	Normal
Psychomotor/mental development	Normal	Normal	Normal	Normal but IQ 20 pts. reduced in disease course[a] Asperger syndrome	Normal	Normal	Normal	Normal	Normal	Normal
AED previous/AED current	–	CBZ/CBZ	CBZ, OXCB/VPA	VPA, OXCB, LTG/VPA + LEV	VPA/VPA	CBZ/No	No	No	VPA/VPA	VPA
Epilepsy phenotype	Asympt.	Epilepsy with GTCS	Epilepsy with GTCS	Dravet syndrome (atypical)	FS+	Epilepsy with GTCS	Epilepsy with GTCS and FS	FS	FS +	FS +

	p.Arg1596His Fam3RH									
ID	II-4	II-6	II-7	II-8	III-3	III-4	III-8	IV-3	IV-4 proband	IV-5
Mutation	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–
Gender	F	F	M	M	M	F	M	F	F	F
Age at investigation	59 y	? adult	51 y	48 y	38 y	30y	14 y	6 y	5 y	5 y
Age at seizure onset/offset	–	infancy	5 y/5 y	–	3 y/5 y	Infancy	3 y/6 y	14 m/14 m	8 m/5 y	14 m/3 y
First seizures type	–	FS	Febrile GTCS	–	Febrile GTCS	Febrile GTCS	Febrile GTCS	Febrile GTCS	Afebrile Tonic (?)	Afebrile CPS evolving to GTCS
FS	No	Yes	Yes	–	Yes	Yes	Yes	Yes	No	Yes
Status Epilepticus	No	No	Cluster of seizures	No	No	No	No	No	No	Yes

Table 1 (*Continued*)

	p.Arg1596His Fam3RH								
Subsequent seizures types	–	–	–	–	–	–	–	Hemiconvulsions left-sided, GTCS	CPS, GTCS
Interictal EEG	ND	ND	ND	ND	ND	Normal	ND	First normal, normal or focal on right	Focal spike-waves
Neuroimaging	ND	ND	ND	ND	ND	Normal (CT)	ND	Normal (MRI)	ND
Clinical examination	Normal	Normal	Normal	Normal		Normal	Normal	Normal	Normal
Cognitive development	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
AED previous/AED current	–	–	–	–	–	–	–	VPA/LEV	VPA
Epilepsy phenotype	Asympt	FS	FS	Asympt	FS	FS plus	FS	Epilepsy with focal seizures	Panayiotopulos syndrome

[a] Wechsler test.
Abbreviation: ND – not done; GTCS – generalized tonic-clonic seizures, CPS – complex partial seizures, FS – febrile seizures, FS+ – febrile seizures plus; AED – anti-epileptic drugs, CBZ – carbamazepine, OXCB – oxcarbazepine, VPA – valproic acid, LTG – lamotrigine, LEV – levetiracetam.

was not available. Patients' II-1 and III-1 DNA was unavailable for the analysis.

2.2.3. *Family 3 (Fam.3RH)*

Two girls IV-4 and IV-8, in this family, had come to medical attention independently from each other, so at that time they were regarded as probands of two unrelated families. However it turned out that they belonged to the same four-generation family with additional 7 clinically affected and 2 asymptomatic members being p.Arg1596His mutation carriers.

The patient IV-4 was labelled as a proband for this family. She was 5-year-old girl with uneventful prenatal history and normal psychomotor development. At the age of 8 months she experienced her first afebrile tonic seizure lasted about 3 min, followed by clouding of consciousness lasting about 1 h. The next seizure, also afebrile, occurred 4 months later. It was motor left-sided seizure with disturbances of consciousness lasting a few minutes. Sleep EEG was normal. Since then, seizures without hyperthermia had repeated every 2 weeks, always involving the left side of the body, sometimes with secondarily generalization. At that time she was diagnosed with cryptogenic focal epilepsy and VPA treatment was introduced. After a few months of remission seizures, mostly afebrile, came back. In fact, only two seizures at the age of 2 and 4 years of age were associated with fever. She had never experienced status epilepticus, however, once developed the cluster of three seizures. Her interictal EEG records were either normal or showed single sharp waves/sharp and slow waves in both centro-parietal regions, on the right side, predominantly. The MRI scan was normal. Seizures seized after introduction of LEV at the age of 3 years as alternating monotherapy. Her cognitive development was within normal range (IQ = 81 at the age of 4.3 years). Proband's older sister (IV-3) with normal IQ had one FS. Their father (III-3) had a few simple FS, whilst proband's paternal grandmother (II-4) was asymptomatic.

The patient IV-8 was 5-year girl with uneventful prenatal history and normal mental development. She was diagnosed with Panayiotopoulos syndrome. At the age of 14 months she developed afebrile complex partial seizure with autonomic signs evolving to tonic–clonic seizures lasting few minutes. Since then, she had numerous febrile and afebrile clonic seizures with vomiting, lasting from 10 min to 2 h. Once, during febrile status epilepticus lasting for 2 h she experienced long apnoea (required intubation) and right-sided Todd's paresis. At times, she also complained on paroxysmal blindness lasting a few minutes. The MRI scan was normal. EEG interictal showed spike-waves complex in right central area. After introduction of VPA at the age of 3.3 years, the patient developed no more seizures. Her mother (III-4) and maternal grandmother (II-6) had FS in their early childhood.

2.3. *Molecular analysis*

Molecular analysis was performed on genomic DNA extracted from subjects' venous blood isolated using Genomic Maxi AX kit (A&A Biotechonology).

Probands were screened for the *SCN1A* gene point mutations and exons rearrangements. The direct sequencing of all 26 exons of the gene was performed. Exons were amplified by

PCR with specific intronic primers (data available on request). PCR products were sequenced using ABI BigDye v.3.2 terminator sequencing kit (Applied Biosystems). To exclude large-scale rearrangements of the gene multiplex ligation-dependent probe amplification (MLPA) was performed using the commercially available MLPA, P-137A2 SCN1A kit (MRC-Holland).

Sequence data were analyzed using MutationSurveyor V3.24 software (SoftGenetics) in comparison to reference sequence NM_001165963.1 (NCBI RefSeq; http://www.ncbi.nlm.nih.gov). The identified variants were labelled according to numbering of the longest transcript of *SCN1A*, cDNA accession number AB093548.

MLPA data were analyzed using the GeneMarker V1.8 software (SoftGenetics) with standard parameters.

Proband's parental and relatives samples were tested by direct PCR amplification and DNA sequencing of the *SCN1A* exon 25.

The impact of discovered p.Arg1596 residue substitutions on the protein structure and functional changes were analyzed with the PolyPhen-2/HumVar model software (http://genetics.bwh.harvard.edu/pph2) [12] and Mutation Taster Software (http://www.mutationtaster.org) [13] and Align-GVGD (http://agvgd.iarc.fr), using the A-GVGD method scoring missense substitutions against the range of variation present at their position in a multiple sequence alignment [14].

3. Results

Two types of missense substitutions at the p.Arg1596 residue of the Nav1.1 – p.Arg1596His and p.Arg1596Cys (c.4787G > A and c.4786C > T) have been identified as recurrent mutations among patients of Polish origin referred for *SCN1A* analysis and with confirmed mutation in the gene. This was one of the four such *SCN1A* mutations we described in this cohort (88 subjects), and the only one, which was heritable and giving such phenotypic intra-/interfamilial heterogeneity in our material. The remaining mutations p.Arg712*; Ex12 c.2134C > T (3.4% probands'), p.Arg946His; Ex15 c.2837G > A (2.3% probands'), p.Lys1846Serfs*; Ex26 c.5536_5539del; (3.4% probands') all caused the DS phenotype and raised *de novo* (confirmed in 75% of probands).

Prediction analysis of the p.Arg1596 substitutions' functional effect, using PolyPhen2/HumVar algorithm revealed, that both identified variants are *probably damaging* (score 1.0), as well as another known substitution – p.Arg1596Leu. These results were confirmed by Mutation Taster prediction (model: simple_aae) and all mutations were classified as *disease causing* (prob: 0.999). However the Grantham score (GS, range 0.0–215) differs between different substitutions, for Arg > His GS = 28, Arg > Cys GS = 180, Arg > Leu GS = 102 reflecting the degree of differences in physico-chemical characteristic of exchanged amino acids. Both identified substitutions were described as pathogenic in Human Gene Mutation Database [HGMD Professional]; they also cosegregate with FS/Epilepsy phenotypes in families under consideration.

The substitution p.Arg1595Cys was identified in the Exome Variant Server database (EVS, http://evs.gs.washington.edu/EVS) with frequency in European cohort 7.7×10^{-5} (1.1×10^{-4} for European American cohort).

Mutations of the p.Arg1596 were identified in three families. In one family the substitution p.Arg1596Cys (proband diagnosed with atypical DS; Fam1RC III-2) was found and in two families p.Arg1596His (at the beginning in three, but detailed data and medical/familial history analysis, allowed to merge two of them), where probands were diagnosed with FS+ (Fam2RH, II-4) and focal epilepsy (Fam3RH IV-3). In all cases mutation was heritable, and we were able to correlate the presence of mutation and FS/epileptic phenotypical features for the most family members. We observed the general tendency to evolution from asymptomatic carriers to symptoms developing patients in the following generation within all families (Fig. 1).

4. Discussion

We have identified substitutions at the p.Arg1596 residue as one of the few recurrent mutation in the *SCN1A* gene in Polish DS/GEFS+ patients' cohort. Two different nucleotides substitutions in the 25th exon of the *SCN1A* gene, c.4787G > A and c.47865C > T were identified as a causative of missense mutations at the p.Arg1596 residue – p.Arg1596 His and p.Arg1596Cys of the Nav1.1 protein respectively. The wild type of p.Arg1596 is well-conserved amino acid across evolution (96% homology among mammals) and it is localized in the Nav1.1 D-IV segment at the joint of the transmembrane domain S2 and inner loop S2–S3. The both mutations as well as p.Arg1596Leu substitution were previously reported as causative in cases of the *SCN1A*- related disorders. The p.Arg1596His was described in paternally inherited GEFS+ syndrome [15] and p.Arg1595Cys in *de novo* DS, GEFS+ and focal epilepsy syndromes [1,16,17]. Another substitution, not identified among our patients p.Arg1596Leu (c.4787G > T), was described *de novo* as causative for DS [15]. In the published case of inherited p.Arg1596His substitution, no detailed clinical data were provided, so we are not able to analyze the possible phenotypic differences between carriers. Interestingly, the substitution p.Arg1595Cys, among the others, was identified in the Exome Variant Server (EVS), even reported as pathogenic for epilepsies [18]. This finding may indirectly indicate that this substitution may be not fully penetrant, and its different expressivity depends on additional modifying genetic factors. Of course, we must be aware, that EVS was created for identification of genes not related to epilepsy, so the recruited subjects were not necessary free of undiagnosed/unreported seizures (EVS, http://evs.gs.washington.edu/EVS). However, data we have obtained for all analyzed families show intrafamilial variability in clinical course of disease, what indicates the reduced penetrance of Arg substitutions in position 1596 (asymptomatic carriers) and genetic modifiers role in final phenotype. The influence of the single nucleotide polymorphisms, in two others ion channel coding genes *SCN9A* and *CACN1A*, on the disease course of the *SCN1A* mutation-positive DS patients has already been postulated [8,9]. Additionally, there are data indicating the *GABRG2* polymorphisms association with susceptibility to FS [19] and *SCN1A*, *SCN2A* in epilepsy susceptibility and drug response (however controversial) [20,21]. In this context, the work of Klassen et al. is worth to be mentioned too, as it shows that

even in monogenic form of epileptic syndromes the individual patient's ''channotype'' – the ion channels variation profile contributes to the excitability phenotype [22].

From the clinical point of view, the epilepsy phenotypes in presented three unrelated families with Arg1596Cys/His mutations were within the spectrum of GEFS+ [23] showing phenotypic intra- and interfamilial variability. The phenotypes of 20 mutation carriers range from asymptomatic cases, through febrile seizures, febrile seizures plus, epilepsy with GTCS either preceded or not by FS, focal epilepsy, atypical Dravet syndrome and Panayiotopoulos syndrome.

The most interesting, but also the most controversial in terms of clinical diagnosis was the proband (III-2) in Fam.1RC. The authors considered whether GEFS+ should have been diagnosed or borderline DS. Epilepsy course might have suggested DS. The first psychological exam was done at 4.5 years with IQ = 120, and the next – 140, during seizures remission at the age of 6 years. Observed facts provide evidence, that existing epilepsy influenced on cognitive regression (above 1SD) in patient with high level at starting point. OXCB and LTG were given during short period of time, thus probably not having negative impact on ex post detected cognitive regression. Asperger syndrome features and dyspraxia appeared in this period additionally. There are a few descriptions of children with Dravet syndrome in whom IQ maintained within normal range after achieving seizure remission [16,24,25]. Having this multifaceted data, the authors classified his clinical phenotype as borderline Dravet syndrome. Similar clinical issues in diagnosis one may have in very young, normal development patients with recurrent seizures and confirmed mutations in *SCN1A*. DS is usually diagnosed in these patients, without full confidence of developmental regression appearance in the future. There is a chance that future will reshape diagnosis stated today, especially that EEG findings show two patterns: focal and generalized epilepsy in this patient.

The proband of Fam.3RH (IV-4) suffered from focal epilepsy with mostly afebrile seizures, not heralded by febrile seizures. Based on epilepsy phenotype alone she had been for the first time presented to our clinic with diagnosis of focal cryptogenic epilepsy. However analysis of the family history, which revealed FS and FS plus in proband's relatives prompted us to consider channelopathy as the background of seizure disorders. Molecular diagnosis revealed *SCN1A* mutation which confirmed our suspicion of genetic focal epilepsy.

In fact, patients with *SCN1A*-related partial epilepsies were described quite rare few years ago. However Lossin et al. found that patients with focal epilepsies might constitute 1.1% among the subjects with epilepsy caused by *SCN1A* mutations [26]. This indicates that the full spectrum of *SCN1A*-related epilepsies include both generalized and partial epilepsies.

Another patient from Fam.3RH (IV-8) with normal intelligence and neuroimaging was presented with constellation of both afebrile and febrile long-lasting autonomic and complex partial seizures which let us to consider diagnosis of Panayiotopoulos syndrome (PS). The girl also complained from recurrent ictal blindness, which could be visual seizures occurring in few percent patients with PS [26]. Autonomic symptoms she suffered were mostly emetic and paleness, but once during status epilepticus she experienced long-lasting apnoea required even intubation. In fact, despite benign course of PS in mostly of patients, cardiorespiratory arrest with potentially fatal outcome has been reported in four out of around 1000 cases [26]. It is worth to stress, that recognition of molecular defect in *SCN1A* gene in our patient allowed avoiding treatment with CBZ and LTG, which might have had deleterious effect on the disease course, especially on autonomic symptoms. Once she experienced postictal Todd's paresis which has been observed in some patients with both symptomatic and idiopathic focal epilepsies, *e.g.* rolandic epilepsies [27]. So far we have not found description of postictal paresis in a child with PS. However, it is well known, that there is link between PS and rolandic epilepsy and some clinical and EEG signs of the latter epileptic syndrome can occur in less typical Panayiotopoulos syndrome, as well as atypical absences, atonic and inhibitory seizures [27]. In EEG trace we have found only extra-occipital spikes which do not exclude diagnosis of PS, as quite typical focal and multifocal sharp waves are located in the occipital lobe in about two-thirds of the cases [27]. PS is very rarely described among patients with *SCN1A* mutation. Cordelli et al. [28] have found no *SCN1A* mutation among 10 children with PS, and then even advised against routine screening of the *SCN1A* gene in patients with focal autonomic seizures triggered by fever. In our case, sensitivity to fever and positive family history with FS strongly suggested to perform analysis of this gene. PS and FS due to *SCN1A* mutation was additionally identified in another patient in the cohort under analysis (unpublished data). Occurrence together the PS and FS (17% of PS patients [29]) may to point on the *SCN1A* mutations as a possible background of symptoms, so following Grosso et al. [30], we suggest that the molecular analysis of the *SCN1A* gene might be taken in account and performed in such cases as a helpful during treatment consideration.

It is worth to state that all p.Arg1596 mutations carriers had normal intelligence, and epilepsy phenotypes of probands and their relatives, although different, had quite benign clinical course when being diagnosed as sodium channelopathy and afterwards treated properly. Modification of previous drug regimen was needed in a few cases, because patients had been treated with CBZ. Some of them had not achieved remission of seizures on CBZ, but the others had. How could it be explained may be their epilepsy was benign enough, that CBZ did not make much harm, or the lifetime of dysfunctional sodium channel was over at certain age of a patient. Dlugos et al. for example, presented a 6-year-old boy with *SCN1A* p.Arg1596Cys substitution, who was temporary managed, among the others, with sodium channel blockers (CBZ and LTG). Despite this his cognitive development remained normal. He finally achieved seizures remission on VPA monotherapy [16].

To conclude, the spectrum of *SCN1A*-related phenotypes in set of patients with p.Arg1596 mutations ranged from asymptomatic cases, through FS and FS plus to epilepsies with and without febrile seizures and epileptic syndromes such as DS and Panayiotopoulos syndrome. Phenotypes of epilepsies differ among patients displaying both focal and generalized epilepsies. Analysis performed for families with different substitutions at this same protein residue indicates that the other than just the single *SCN1A* mutation must be involved in phenotype of broad-spectrum development. Possibility of occurrence DS and milder form among relatives (and such

results are more common with more cases being analyzed), shows how difficult may be prediction of the disease course – the type of epileptic syndrome, but also presence of additional signs as ataxia, migraine or neuropsychiatric diseases (*e.g.* Asperger syndrome). This also makes the genetic counselling for mutation's carrier much more complicated even in familial cases of *SCN1A*-related disorders.

Conflict of interest

No conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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