

## Can the p.Thr1174Ser Mutation in SCN1A Gene Shape Genetic Background in Epileptic Encephalopathies?

Dorota Hoffman Zacharskaa<sup>1</sup>, Iwona Terczyńska<sup>2\*</sup>, Paulina Górka-Skoczylasa<sup>1,2</sup>, Anna Winczewska Wiktor<sup>3</sup>, Tomasz Mazurczak<sup>2</sup>, Jolanta Góral<sup>4</sup>, Agnieszka Charzewska<sup>1</sup>, Kinga Duszyc<sup>1</sup> and Elżbieta Szczepanik<sup>2</sup>

<sup>1</sup>Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland

<sup>2</sup>Clinic of Neurology of Child and Adolescents, Institute of Mother and Child, Warsaw, Poland

<sup>3</sup>Paediatric Neurology of Developmental Age Clinic, Poznan University of Medical Sciences, Poznan, Poland

<sup>4</sup>Department of Developmental Neurology and Paediatrics, SPS ZOZ-Zdroje, Szczecin, Poland

\*Corresponding author: Iwona Terczyńska, Clinic of Neurology of Child and Adolescents, Institute of Mother and Child, Kasprzaka 17A, 01 211 Warsaw, Poland, Tel: +48 22 32 77 307; Fax: +48 22 32 77 130; E-mail: iwona.terczyńska@onet.eu

Received date: Feb 06, 2016; Accepted date: Mar 03, 2016; Published date: Mar 10, 2016

Copyright: © 2016 Zacharskaa DH, et al., This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

Dravet Syndrome (DS) and Genetic Epilepsy with Febrile Seizures plus (GEFS+) are very often caused by mutations in the SCN1A gene. These mutations also have been identified in families with migraine phenotypes, supporting the link between migraine and epilepsy. The SCN1A substitution p.Trp1174Ser has been reported as a cause of familial migraine and familial mixed phenotypes with seizures / hemiplegic migraine. We present this mutation as a causative factor of familial GEFS+ syndrome, but also as a factor potentially changing the phenotypes of the epileptic encephalopathies caused by mutations in the SCN1A, ARX or PCDH19 genes. Substitution p.Trp1174Ser was identified in five probands clinically diagnosed as spectrum of GEFS+ or DS. As it has not been regarded as significant for the epileptic encephalopathy, they underwent additional testing according to the revised phenotypes. Probands were finally diagnosed with GEFS+ (p.Trp1174Ser SCN1A mutation only) and epileptic encephalopathies: DS (p.Arg712\* and p.Arg1245\* in SCN1A), Epilepsy and Mental Retardation Limited to Females (p.Asp155Tyr in PCDH19) and atypical West Syndrome (del79nt IVS4/Ex5 in ARX). This study indicates a complex involvement of some SCN1A mutations in epilepsies / epileptic encephalopathies also as a modifying factor with the SCN1A, PCDH19, ARX and possibly mutations in other genes. In cases with atypical or "plus" course or more severe course the possible involvement of other genetic factors should always be considered. Additional modifiers identification may influence on clinical prognosis, patient management and genetic counselling.

**Keywords:** Epileptic encephalopathies; Phenotypic heterogeneity; SCN1A mutations; Genetic modifiers

### Introduction

Mutations in the *SCN1A* gene, encoding the  $\alpha 1$  subunit (Nav1.1) of neuronal voltage-dependent sodium channel are well recognized as underlying spectrum of epilepsy syndromes, ranging from simple febrile seizures / genetic epilepsy with febrile seizures plus type 2 (FS/GEFS+), featuring mild symptoms, to severe epileptic encephalopathies such as Dravet syndrome (DS). Less commonly, they are identified in other epilepsy syndromes. The *SCN1A* is also the third gene, apart from *CACNA1A* and *ATP1A2*, involved in the expression of familial hemiplegic migraine (FHM types 3, 1 and 2 respectively) [1]. Identification of families with migraine phenotypes due to mutation in the *SCN1A* supports the link between migraine and epilepsy and their common molecular pathways. Since 2005, when the *SCN1A* has been identified as another gene involved in FHM pathogenesis [2], a bunch of mutations, all missense substitutions, have been associated with that phenotype [HGMD, Human Gene Mutation Database Professional v.2014.4]. Identified mutations are responsible for different phenotypes, including pure FHM, migraine with / without aura and mixed phenotypes with seizures / migraine. The p.Thr1174Ser substitution was described in a few families presenting heterogeneous phenotypes: migraine with and without aura accompanied by seizures [3], myoclonus and ataxic migraine

syndrome [4], migraine and severe myoclonic epilepsy with global developmental delay [5], myoclonic astatic epilepsy (MAE) [6], but also juvenile myoclonic epilepsy (JME) [7]. Various clinical manifestations in patients carrying the same mutations, suggest that this variability is likely to a result from modifying effects of other genetic or environmental factors. Functional studies of the p.Thr1174Ser substitution effect, performed by Cestele et al. [8] showed its possible dual nature. The mutation in inhibitory interneurons may cause a loss of their function being epileptogenic or gain of function lead to migraine phenotype. The switch of functional effect - from function gain to loss - may be the issue of modulating genetic factors influence. Specific phenotype probably results from the interactions of a couple of genes modifying the functional properties of mutated Nav1.1. [8] However, we can also think about mutations in the *SCN1A* as a part of genetic background - modifiers in other epileptic syndromes.

We report five families presenting different and intrafamilial heterogeneous epileptic phenotypes accompanied by migraine, GEFS+, DS, Epilepsy and Mental Retardation Limited to Females (EFMR) of non-classical phenotype and West Syndrome (WS) with atypical course. Clinical diagnosis of all patients was molecularly confirmed by identification of pathogenic mutation in appropriate for the syndrome gene: *SCN1A*, *PCDH19* or *ARX*, respectively.

Mutations in the *PCDH19* are one of the common causes of epileptic encephalopathy, identified in female patients clinically

diagnosed with DS without mutations in the *SCN1A* [9,10]. There is a group of other genes giving DS-like phenotype, e.g. mutations in *CHD2* recently described, but *PCDH19* is still the first choice in girls [11]. The *PCDH19* encodes the protein protocadherin-19, involved in cell adhesion. It is expressed during the brain development and plays important role in neuronal migration and formation of synaptic connections [12]. The *ARX* encodes the Aristaless-related homeobox protein acting as a transcription factor modulating cerebral development and patterning. Mutations in this gene have been associated with syndromes exhibiting variable phenotypes, including idiopathic WS [13]. WS clinical picture differs significantly from *SCN1A*-related disorders; however, the single case of mutation in *SCN1A* was described as causative for infantile spasms [14].

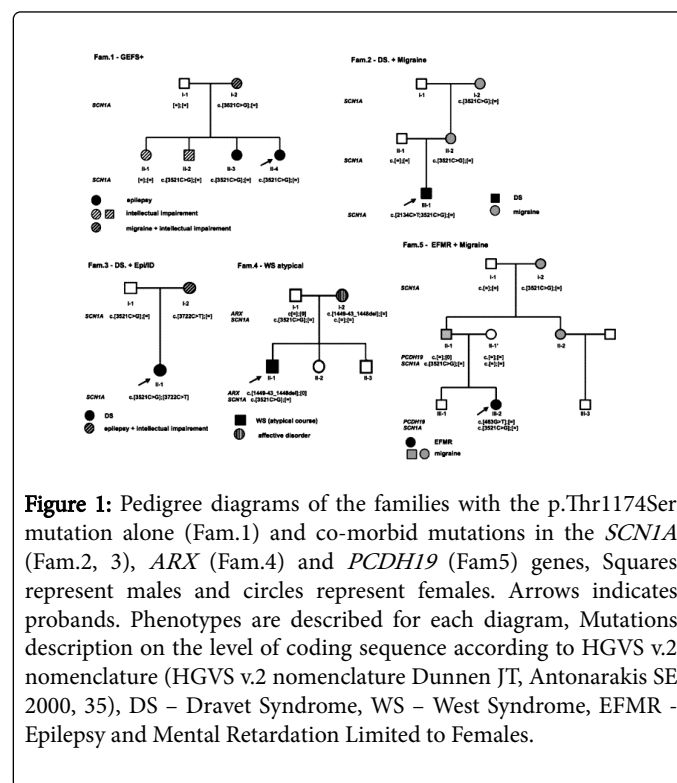
All probands were also carriers of heterozygous mutation in the *SCN1A* – p.Thr1174Ser, which seems to modify the patients' main phenotype. This substitution by itself has been causative for GEFS+ phenotype in one family and migraine in probands' relatives. The purpose of our report is to present this mutation as a possibly causative factor of familial GEFS+ syndrome, but also as a factor potentially changing the phenotypes of the epileptic encephalopathies, caused by other mutations in the *SCN1A*, *ARX* or *PCDH19*.

## Material and Method

### Subjects

Five families (Figure1) with different types of epilepsy / epileptic encephalopathies were included into the study due to the presence of the p.Thr1174Ser (c.3521C>G) substitution in the *SCN1A*. Blood samples were collected from 16 affected individuals (5 probands) and their 6 asymptomatic relatives; this substitution was hereditary in all families. All families' probands were among the patients referred for the *SCN1A* testing and clinically diagnosed with GEFS+ spectrum or DS (typical and atypical). This patients group included 250 probands representing the same number of families. Additional genes analysis

was performed in two epileptic encephalopathy cases, leading to *PCDH19* and *ARX* mutations identification.



**Figure 1:** Pedigree diagrams of the families with the p.Thr1174Ser mutation alone (Fam.1) and co-morbid mutations in the *SCN1A* (Fam.2, 3), *ARX* (Fam.4) and *PCDH19* (Fam.5) genes, Squares represent males and circles represent females. Arrows indicates probands. Phenotypes are described for each diagram, Mutations description on the level of coding sequence according to HGVS v.2 nomenclature (HGVS v.2 nomenclature Dunnen JT, Antonarakis SE 2000, 35), DS – Dravet Syndrome, WS – West Syndrome, EFMR - Epilepsy and Mental Retardation Limited to Females.

Finally (Table 1), probands were diagnosed with GEFS+ (1 subject, Fam.1), DS (2 sub., Fam. 2, 3), WS with atypical course (1 sub., Fam. 4) and EFMR (1 sub., Fam. 5). After mutations identification the clinical data were re-evaluated.

	Proband 1/Fam.1	Proband 2/Fam.2	Proband 3/Fam.3	Proband 4/Fam.4	Proband 5/Fam.5
Genotype (gene, mutation)	<b>SCN1A</b> c.[2134C>T;3521C>G];[=] p.[Trp1174Ser];[=]	<b>SCN1A</b> c.[2134C>T;3521C>G];[=] p.[Arg712*;Trp1174Ser];[=]	<b>SCN1A</b> c.[3733C>T];[3521C>G] p.[Arg1245*]; [Trp1174Ser]	<b>SCN1A</b> c.[2134C>T;3521C>G];[=] p.[Trp1174Ser];[=] <b>ARX</b> c.[1449-43_1448del];[0] p.[?];[0]	<b>SCN1A</b> c.[2134C>T;3521C>G];[=] p.[Trp1174Ser];[=] <b>PCDH19</b> c.[463G>T];[=] p.[Asp155Tyr];[=]
Gender	F	M	F	M	F
Age at investigation	6 y.	17 y.	11 y.	21y.	10 y.
Diagnosis	<b>GEFS+</b>	<b>Dravet syndrome</b>	<b>Dravet syndrome</b>	<b>Atypical syndrome</b> <b>West</b>	EFMR
Family history (Pedigrees Figure 1)	intellectual impairment and migraine in mother; epilepsy in sister; intellectual impairment in sister and brother	migraine in mother and maternal grandmother	intellectual impairment and epilepsy in mother	emotional disturbances in mother	migraine in father, father's sister and grandfather
Age at onset	3 mo.	8 mo.	7 mo.	3 mo.	7 mo.
First seizure	tonic-clonic, febrile seizures	hemiclonic during infection	febrile seizures (type unknown)	vaccination, loss of consciousness	tonic-clonic during infection

Subsequent seizure types	tonic-clonic, hemiclonic, tonic with cyanosis	tonic-clonic, hemiclonic, myoclonic, complex seizures, tonic	tonic-clonic, hemiclonic, tonic with cyanosis, absence, myoclonic	infantile spasms, atonic, hemiclonic, myoclonic, tonic-clonic	tonic with apnoea, tonic-clonic
Seizure clusters	Yes	Yes	Yes	Yes	Yes
Status epilepticus	No	Yes	Yes	Yes	Yes
Acute encephalopathy	No	No	No	Yes	Yes
Seizure induced by	-	CBZ, LTG, VGB, TPM, GBP	CLN, VGB, OXC, LTG	VGB, TPM	LTG, VGB
Improvement with	VPA	VPA, CLB	LEV	VPA, BZD, LEV	STR
Remission age	5 mo.	no	no	12 y.	8 y.
EEG	focal changes and then normal	generalized changes, slowing background	generalized and focal changes, slowing background	hypsarrythmia, then generalized changes	focal and generalized changes, then normal
Neurological exam	normal	ataxia, tetraparesis	ataxia	tetraparesis	ataxia
Development	normal at onset; intellectual impairment at the age of 6 years	normal at onset; regression from the age of 2 years; severe intellectual impairment now	normal at onset; significant regression and autism at the age of 2 years	normal at onset; delayed in the first year of life; acute regression related to encephalopathy at the age of 6 years	normal at onset; start delayed in the second year of life; mild intellectual impairment now
Comments	Intellectual impairment is not related to SCN1A mutation; caused probably by other familial factors	Severe disease course was partially related to ineffective treatment, the presence of the <i>SCN1A</i> p.Trp1174Ser has no influence of phenotype as is monoallelic and localised 3' to premature STOP codon	Severe phenotype with fast progressing intellectual impairment and autism might be related to coexistence of two mutations in <i>SCN1A</i> gene	Hemiclonic seizures triggered by fever and acute encephalopathy episode and treatment response might be related to additional mutation with <i>SCN1A</i>	Severe EFMR phenotype, treatment response and appearance of acute encephalopathy might be related to additional mutation in <i>SCN1A</i>

**Table 1:** Characterization of the probands with p.Thr1174Ser substitution with/without additional pathogenic mutations in *SCN1A* or other gene, EFMR – Epilepsy and Mental Retardation Limited to Females, CBZ – Carbamazepine, LTG – Lamotrigine, VGB- Vigabatrin, TPM - Topiramate , GBP- Gabapentin, OXC – Oxcarbazepine, VPA – Valproic acid, CLB - Clobazam , LEV – Levetiracetam, STP – Stiripentol.

GEFS+ inclusion criteria: familial occurrence of classic febrile seizures, febrile seizures plus (with onset or persisting after 6 years of age, which are followed by afebrile tonic-clonic seizures), severe epileptic encephalopathy and other types of generalized or focal seizures [15].

Inclusion criteria for Dravet syndrome: normal development before onset, first seizures start in the first year of life in the form of generalized or unilateral febrile clonic / tonic-clonic seizures, secondary appearance of refractory different types of seizures (myoclonic, complex partial seizures and absences), often with photosensitivity, EEG often normal at first, but later characteristically shows generalized spike-wave activity, psychomotor development stagnates around the second year of life, and affected individuals show subsequent mental decline and other neurologic manifestations [16].

Phenotypes of epilepsies were verified and classified according to International League against Epilepsy (ILAE) classification system [17,18].

### Ethics statement

The study was approved by the Ethics Committee of the Institute of Mother and Child, Warsaw (Poland). Written informed consent from

all participants (parents and adult patients) was a criterion for the inclusion in the study.

### Genetic testing

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

Analysis of the all relevant genes – *SCN1A*, *PCDH19* and *ARX* – was performed by direct sequencing (Sanger method). The exons with exon / intron boundaries were sequenced with specific intronic primers (primers sequence, PCR conditions available on request). The ABI BigDye v.3.2 terminator sequencing kit (Applied Biosystems) was used. The multiplex ligation-dependent probe amplification (MLPA) was performed using commercially available MLPA probemixes: P-137A2 *SCN1A* and P330-A1 *PCDH19* (MRC-Holland) for rearrangements analysis in the *SCN1A* and *PCDH19* genes.

Sequence data were analysed using Mutation Surveyor V3.24 software (Soft Genetics LLC.) in comparison to appropriate reference sequences NM\_001165963 *SCN1A*; NM\_001184880 *PCDH19*, NM\_139058 *ARX* (all sequences according to GRCh37 Human Genome release). Variants were labelled according to numbering of the

longest transcript of cDNA accession number AB093548 (NCBI Ref Seq) in the case of the *SCN1A* gene identification.

MLPA data were analysed using the Gene Marker V1.8 software (Soft Genetics LLC.) with standard parameters.

Proband's parental and relatives samples were tested by direct PCR amplification and DNA sequencing of the appropriate exons in relevant genes (*SCN1A* and *SCN1A/PCDH19* or *SCN1A/ARX*). In two cases of identified coexistence of two mutations in the *SCN1A*, their mono / biallelic localization was analysed. When both mutations (p.Arg1245\* and p.Thr1174Ser) were inherited, inheritance analysis was performed. In the case when one of mutations was de novo (p.Arg712\*), its origin – paternal or maternal was defined by *SCN1A* SNPs analysis. Referring to the dbSNP database the SNPs of the *SCN1A* close to mutations in the exon12 (introns 11 and 12) were selected (data on request).

The impact of discovered missense substitutions on the protein structure and functional changes were analysed with the PolyPhen-2 / HumVar model software and Mutation Taster Software [19,20]. Splice site mutation prediction was performed using Alamut Visual Splicing Predictions software (Alamut Visual v. 2.5, Interactive Biosoftware).

Involvement of the identified mutations in the clinical phenotypes was checked by analysis of the Human Gene Mutation Database Professional (HGMD Professional v.2014.4).

## Results

### Identification of pathogenic mutations

All probands had got heterozygous mutation in the exon 17 of the *SCN1A* c.3521C>G, p.Trp1174Ser.

Based on HGMD data, this mutation has already been reported as pathogenic, and is associated mainly with hemiplegic migraine. However, there is still a question mark over this substitution pathogenicity, as it is also reported in dbSNP database (rs121918799), and in ExAC database with MAF = 0.27 for European Non-Finish population and 0.18 for general analysed population (ExAC). As the mutation p.Thr1174Ser shows variable penetrance, the mutation's carriers were not necessary free of undiagnosed / unreported seizures or migraine (individuals with severe paediatric diseases were excluded from the ExAC data set). We must be aware that this database was created from a variety of large-scale sequencing projects of various disease-specific (no epilepsy project included) and population genetic studies. According to ClinVar Database, the clinical significance of this substitution is also uncertain.

In all patients presented in the paper, c.3521C>G, p.Trp1174Ser substitution was heritable, and could be correlated in some families with migraine phenotype. Analysis of the *SCN1A* in family with GEFS+ (Fam.1), revealed only this one mutation.

Patients with DS had also got truncating mutations, we recognized as main pathogenic in *SCN1A*: c.2134C>T, p.Arg712\* (Fam.2) and c.3733C>T, p.Arg1245\* (Fam.3). Both had already been reported in HGMD as pathogenic and related to Myoclonic Epilepsy of Infancy. The first mutation - p.Arg712\* was a novel one, the second p.Arg1245\* - was inherited from less affected mother. The p.Arg712\* mutation raised de novo on maternal allele and was monoallelic with p.Trp1174Ser (patient's *SCN1A* genotype c.[2134C>T;3521C>G];[=]), mutations p.Arg1245\* and p.Trp1174Ser were biallelic of maternal and

paternal origin respectively (patient's *SCN1A* genotype c.[3733C>T];[3521C>G]).

In female EFMR patient heterozygous, de novo mutation c.463G>T, p.Asp155Tyr was identified in the *PCDH19*. This is a novel mutation, not reported in HGMD. It causes the substitution of highly conservative amino acid localised in extracellular, functional cadherin domain probably influencing the homophilic interactions of *PCDH19* molecules. Substitution functional effect analysis using the prediction algorithms showed high scores, indicating it as a protein function damaging variant (PolyPhen-2, Mutation Taster). The p.Trp1174Ser mutation was in this case of paternal origin (patient's genotype *PCDH19* c.[463G>T];[=], *SCN1A* c.[3521C>G];[=]).

The male patient finally diagnosed with WS with atypical course, was referred twice as having epilepsy of unknown aetiology and later as DS-like. He had got the novel, not previously described mutation in the X-linked *ARX*, deletion of the 79nt in IVS4 and Ex5, c.1449-43\_1448del [HGMD]. This mutation alters the acceptor splice site of exon 5, probably causing the last exon skipping and synthesis of truncated protein (Alamut Visual Splicing Predictions). Mutation in the *ARX* was of maternal and *SCN1A* p.Trp1174Ser of paternal origin (patient's genotype *ARX* c.[1449-43\_1448del];[0], *SCN1A* c.[3521C>G];[=]).

### Clinical features of patients with identified mutations

All probands development was within normal range till disease occurrence, and pregnancy history was not complicated with the exception of proband from the Fam.1, born by caesarean section (Apgar 10), due to pregnancy complicated by diabetes.

#### Case 1: GEFS+ (*SCN1A* p.Thr1174Ser)

GEFS+ family proband (Table1; Figure1, Fam.1 II-4) was the 6-year-old girl, who at the age of 3-5 months presented tonic-clonic, and tonic seizures with cyanosis, and hemiclonic fits, appearing on both sides alternatively. Seizures were related to infections and high fever in initial period, then without fever, clustered, but short lasting (approx. 1 min), ceasing spontaneously. Focal spikes in temporo-parieto-occipital were observed in ictal EEG. Neurological examination, MRI and metabolic tests were within normal range. Seizures ceased under valproate (VPA) treatment. EEG normalization was observed during the following years. Cognitive development regressed from 100 (Brunet-Lezine scale) in the second year of life to 60 points (Leiter test) at the age of 6 years.

Proband's sister (II-3) with normal IQ, developed afebrile generalized tonic – clonic seizures (GTCS) and complex partial seizures (CPS) at the age of 8 months, which were ceased during VPA treatment. Family history revealed also mild intellectual impairment and migraine in proband's mother (I-2), and intellectual impairment without epilepsy in the second sister and brother (II-1, II-2). All mentioned relatives except II-1, were heterozygous *SCN1A* p.Thr1174Ser mutation carriers (Figure 1).

#### Case 2: DS (*SCN1A* monoallelic p.Arg712\* and p.Thr1174Ser)

The proband was 17-year-old male (Table 1; Figure 1 Fam.2 III-1). He has displayed febrile hemiclonic seizures (appearing on both sides alternatively) and GTCS since the age of 8 months. He was treated with phenobarbital (PB), carbamazepine (CBZ), VPA, vigabatrin (VGB),

topiramate (TPM), and lamotrigine (LTG), and clobazam (CLB) in different combinations. Seizures were refractory – long lasting GTCS and CPS were observed several times. Myoclonic seizures occurred after gabapentin (GBP) introduction at the age of 7 years. Seizure worsening was observed during ketogenic diet. Dravet syndrome was diagnosed at the age of 9 years. Parents did not consent for stiripentol (STP) introduction. Tonic seizures during sleep have started since the age of 11 years. His cognitive development has regressed from normal IQ to severe impairment. Neurological examination showed ataxia and mild spastic paraparesis. MRI was normal. Family history analysis revealed migraine without aura in probands' mother (II-2) and maternal grandmother (I-2) who were heterozygous SCN1A p.Thr1174Ser substitution carriers.

### Case 3: DS (SCN1A biallelic p.Arg1245\* and p.Thr1174Ser)

The proband was 11-year-old girl (Table 1, Figure 1 Fam3 II-1). Her first febrile seizures occurred during otitis at the age of 7 months and they were recurrent during the first two years of life. Hemiclonic seizures were appearing on both sides alternatively and tonic-clonic seizures have been observed since the age of 2 years, accompanied by tonic seizures with cyanosis from the age of 4 years and absence seizures from the age of 5 years. Myoclonic seizures occurred when she was 7 years old. Seizures are still polymorphic, very frequent, in clusters and last several hours. VGB, oxcarbazepine (OXC) and LTG triggered seizure appearance. VPA, TPM, CLB, STP treatment did not influence on epilepsy course; mild improvement was achieved after high levetiracetam (LEV) doses introduction, but was observed during one month only. Very rapid development regression was observed at the second years of life; autism with severe mental impairment and ataxia was confirmed when she was 3 years old.

Brain MRI was normal. Generalized series of delta waves, paroxysms of spikes and spike wave complexes (3 – 4 Hz) with slow background activity and focal sharp waves and sharp wave slow wave complexes in the right fronto-temporal region were observed in EEG.

Proband's mother shows intellectual impairment with epilepsy history, but epileptic syndrome has not been precisely diagnosed. She was treated with clonazepam (CLN) and VPA at the age of 1 – 15 years. She is a heterozygous p.Arg1245\* mutation carrier in the SCN1A gene.

### Case 4: WS with atypical course (SCN1A p.Thr1174Ser and ARX c.449-43\_1448del)

Proband, 21-year-old male (Table 1, Figure 1 Fam.4 II-1) is with negative family history for migraine or epilepsy, but his mother (I-2), who was a ARX c.449-43\_1448del mutation carrier suffered from affective disorders. There was no information on any neurological disorders of his father, a heterozygous SCN1A p.Trp1174Ser mutation carrier. At the age of 3 months, the day after DTP+Polio vaccination he experienced short loss of consciousness, with flaccidity and paleness, without seizures. PB treatment was introduced. Patient development had been normal till the age of 7 months, when infantile spasms (IS) occurred with subsequent atonic seizures. Head USG was normal, and hypersarhythmia was observed in EEG. After VPA introduction seizure decrease was observed. Cognitive development and speech regression has been observed since then. Sporadic IS were accompanied by daily hemiclonic seizures (usually in clusters) at the age of 2 years; appearing on both sides alternatively, frequently related to high fever / infection. Generalized paroxysms of spikes and spike wave complexes (3–4 Hz) were observed in EEG. VGB added to existing antiepileptic treatment

caused tonic-clonic status epilepticus. TPM and LTG induced myoclonic seizures. VPA was withdrawn due to pancreatitis. Partial seizure remission was achieved by nitrazepam (NT) introduction at the age of 4 years. Episode of acute encephalopathy, lasting for a week, occurred at the age of 6 years. He was comatose, seizures were not observed. Demyelination process was detected in MRI and generalized background slowing was found in EEG. He was treated with steroids. Laboratory results (including blood tests, cerebrospinal fluid tests) were negative for viral infection and metabolic disorders. Patient general functional status deteriorated – severe mental impairment was diagnosed with subsequent tetraparesis. Full seizure remission was achieved after LEV introduction at the age of 12 years.

The male patient was finally diagnosed with atypical WS.

### Case 5: EFMR (SCN1A p.Thr1174Ser and PCDH19 p.Asp155Tyr)

10-year-old girl (Table 1; Figure 1, Fam5 III-2), developed acute encephalopathy at the age of 7 months, preceded by tonic-clonic seizures. She was comatose for 23 days. Pneumonia and viral encephalitis (her brother was suffering from chickenpox during this period) was diagnosed, even cerebrospinal fluid and blood lab test (general and viral) were negative. Bacterial infection, borreliosis and the most common metabolic disorders were excluded. MRI showed cortico-subcortical atrophy, moderate ventricular enlargement, and hyperintensity on T2-weighted images within the white matter suggesting encephalitis. VPA and PB treatment was introduced; seizures were not observed. Finally, she fully recovered. The next episode of tonic seizures clusters with long-lasting apnoea was observed when she was at the age of 17 months. EEG was normal, intellectual development equalled 83 points, based on Brunet-Lezine scale. TPM was added to previous antiepileptic treatment. Focal seizures clusters lasting several minutes to dozens of hours were observed during the next years (between 23 months of age and 8 years of age). Generalized tonic-seizures were very rare. Antiepileptic and steroids treatment were not effective, LTG and VGB provoked seizures intensity up to status epilepticus. EEG showed focal changes in temporo-parieto-occipital region predominantly in right hemisphere, or generalized, in the form of sharp waves, sharp wave slow wave complexes, and rarely spikes and spike wave complexes. Seizures appearance was not always related to infections, but during high fever with or without seizures her general status was very severe, like in acute encephalopathy. Antiepileptic treatment was modified at 8 year of age – STP was added to VPA and CLB, achieving seizure remission. Patient suffers currently from mild intellectual impairment, which is accompanied by severe ataxia. Mild cerebral atrophy is seen in MRI and EEG findings are within normal range.

The family medical records revealed the migraine history of proband's father (II-1) and grandfather (I-1), both heterozygous SCN1A p.Trp1174Ser mutation carriers. Father's sister also suffered from migraine, but was not available for molecular testing.

## Discussion

Most epilepsies / epilepsy syndromes are characterized by explicit clinical and aetiology heterogeneity. A genetic contribution to epilepsy has been well established through the twin and family studies indicating its mono- and, mainly polygenic / multifactorial character. Recently conducted analysis using the massive parallel sequencing technologies showed, that rare monogenic epilepsies include both

familial and, in the severe cases like epileptic encephalopathies, "sporadic" forms caused by de novo mutations in particular genes. Inter- and intrafamilial heterogeneity of monogenic forms of epilepsy caused by the same mutation indicates other factors involvement – genetic and environmental [21]. The role of such factors in the disease pathogenesis may vary in each patient, what is observed in clinical variability of GEFS+ (GEFSP2) families with *SCN1A* mutations [22]. Genotype–phenotype correlation determination is particularly difficult in cases with different genetic defects coexistence, in which symptoms can be overlapped or endured. The picture is complicated by the fact, that standard diagnostic tests do not detect mutations modifying the main disease course [23]. Such mutations, of small additive effect, may be localized in the same, as a main pathogenic mutation, gene or in the other ones. Such situations seem to be more common than expected since the whole genomic / exomic sequencing (WGS/WES) has been employed in identification of neurogenetic disorders molecular background. The good examples are homozygous / biallelic mutations identification in the *SCN1B* [24,25] and *SCN1A* [26] genes. In the first case patients developed DS, although heterozygous mutations in the *SCN1B* are mainly related to GEFS+ (GEFSP1). Homozygous missense mutations identified in consanguineous families by Brunklaus et al. caused DS and GEFSP2, while heterozygous carriers remained unaffected [26]. Pathological mutations in the *SCN1A* gene were also described as co-morbid with the *POLG* gene mutation [27] and electron transport chain defects [28] resulting in expression of both, mitochondrial disease and DS. This data suggest that multiple gene mutations could alter phenotype in some patients. Many additional factors such as epileptic activity and interictal epileptiform discharges, and antiepileptic treatment, and others environmental factors apart from DNA changes may have impact on phenotypical variability [29]. For example, the significant impact of improper treatment on cognitive development outcome in patient #2 is considered.

Here, we present a group of probands, the p.Trp1174Ser substitution in the *SCN1A* gene carriers, which might be a causative factor of familial GEFS+ syndrome, but also acts as a factor potentially changing the phenotypes of the epileptic encephalopathies caused by other mutations in the *SCN1A*, *ARX* and *PCDH19* genes.

The p.Trp1174Ser mutation caused the GEFS+ in Fam.1 – possible migraine in mother (I-2) and mild epilepsy phenotypes in two children (II-3,II-4). No epilepsy symptoms are observed in boy (II-2), also a mutation carrier, what indicates variable penetrance of this substitution. Intellectual impairment was observed in three subjects of this family, too – mutation's carriers (I-2, II-2) and non-carrier (II-1). That is why it seems this feature should be considered independently from epilepsy symptoms. In two families (Fam.2 and 3) substitution coexisted with truncating *SCN1A* mutations. In the Fam.2 p.Trp1174Ser substitution alone probably caused migraine in proband's mother and grandmother (I-2, II-2). In proband (III-1) it was localised on maternal chromosome 3' in cis to main pathogenic mutation p.Arg712\*, and probably had no influence on the final phenotype. The severe disease course of this patient was rather related to improper treatment before the molecular diagnosis. Different situation was observed in Fam.3. Mutations p.Trp1174Ser and p.Arg1245\* were biallelic. The truncating mutation was heritable, but in proband's mother (I-2) caused less severe phenotype. Proband's clinical picture indicates that missense substitution presence had got significant impact on the disease course. Patient showed acute and severe development regression in the second year of life and very early autism onset. In children with typical DS such symptoms are developed gradually [21] and significant IQ decrease is observed in

longer period of time, usually between the 4 to 6 years of life [30]. This patient showed extreme treatment resistance. During the 11 years of treatment no remission was observed. This might be related to the presence of two trans *SCN1A* mutations of course, although we cannot exclude partial consequence of antiepileptic drugs use contraindicated in DS patients.

From clinical point of view, we found cases 4 (Fam.4 II-1) and 5 (Fam.5 II-1) the most interesting among presented group of patients. Probands were diagnosed with epileptic encephalopathies due to mutations in the other genes - *ARX* and *PCDH19*. However, both of them presented symptoms typical of mutations in *SCN1A* gene, too. The patient with WS developed the first event after vaccination, what is rather typical for children with *SCN1A* mutations [31]. Subsequent infantile spasms and atonic seizures appeared after several months. At the age of 2 years gradual WS remission was observed, but the hemiclonic seizures triggered by high fever / infections started. The presence of such seizures, as well as adverse or no effect to the usually used in WS treatment, may also indicate the significant role of the *SCN1A* mutation on final phenotype expression. The case of female patient harboring both *PCDH19* and *SCN1A* mutations is in fact very complex one. Her phenotype shares symptoms of both EFMR and DS. Probably, that is why she was twice independently referred for genetic diagnosis (once as DS, and the other time as a suspicion of having *PCDH19*). Her epilepsy course with clusters of focal seizures could have suggested the EFMR phenotype, as focal seizures clusters are less characteristic for Dravet syndrome, whilst severe ataxia she developed later on is rather related to DS phenotype [32].

Additionally, WS and EFMR probands suffered from the acute encephalopathy episodes. Its occurrence in children carrying the *SCN1A* mutations has already been reported [33,34]. It may be a very significant complication in DS, but has been also described in previously healthy children, as well as in patients diagnosed with partial epilepsy or febrile seizures, who were carriers of such mutations [32,34-36]. The presence of the p.Trp1174Ser substitution in the *SCN1A* might contribute to acute encephalopathy occurrence in described patients, although in different pathomechanism in each of them, what has been confirmed by the various MRI data (demyelination in WS vs. cortico-subcortical atrophy in EFMR). Various clinical outcomes might be dependent on main mutation type or other factor not known for us, yet.

Migraine was recognized in six relatives of our three patients. It is worth to add that the link between migraine (or broadly meaning 'headaches') and epilepsy is very complicated and beyond the role of *SCN1A*. There are three types of headache-related seizures according to the current ICHD-2 so far: (1) migraine-triggered seizure (migralepsy), (2) hemicrania epileptica and (3) post-ictal headache. Parisi et al. [37] has shown a new view on this topic. According to these Authors, a new entity should be introduced into forthcoming ICHD-3 classification i.e. ictal epileptic headache, where a headache might be a sole manifestation of epileptic seizure or even non-convulsive status epilepticus. So, we could only speculate if some of them being mutation carriers may have epileptic seizures recognized as migraine.

Our data confirm possible involvement of more than one genetic factor as a molecular background in observed phenotype, beside the reduced penetrance or specific mutations variable expressivity. In epileptic encephalopathies cases with atypical or "plus" course the possible involvement of other genetic factors should always be considered. How complex may be influence of molecular factors on

phenotype shows growing evidence that the role of *SCN1A* is beyond the spectrum of GEFS/DS. As Kasperaviciute et al. [38] showed in the GWAS study with 1018 patients, *SCN1A* was associated with mesial temporal lobe epilepsy with hippocampal sclerosis with febrile seizures. More recently, meta-analysis of combined genome-wide association data from 12 cohorts of individuals with epilepsy and controls made by ILAE Consortium on Complex Epilepsies suggests that specific loci can act pleiotropically, raising risk for epilepsy broadly, or can have effects limited to a specific epilepsy subtype which would be the subject of further investigation [39].

Taking this into account, it would be worth considering how wide the genetic diagnosis should be performed in patients whose clinical picture is beyond established epilepsy criteria? Genetic analysis widening should also be considered in families with variable phenotypes, in cases of unexpected antiepileptic drug treatment response and in cases with acute encephalopathies. Additional modifiers identification may influence on clinical prognosis, patient management and genetic counselling. The implications for the latter in patients with 'additional genetic hits' are really complex and this should be considered in the incoming 'NGS era'.

### Conflict of interest

The authors have no conflicts of Interest.

### Acknowledgement

This work was supported by grants from the MSHE/NSC NN407054439 and 800/N-ESFEuroEPINOMICS/10/2011/0.

### References

- Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, et al. (2011) Genotype-phenotype associations in SCN1A-related epilepsies. *Neurology* 76: 594-600.
- Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, et al. (2005) Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet* 366: 371-377.
- Cestèle S, Scalmani P, Rusconi R, Terragni B, Franceschetti S, et al. (2008) Self-limited hyperexcitability: functional effect of a familial hemiplegic migraine mutation of the Nav1.1 (SCN1A) Na<sup>+</sup> channel. *J Neurosci* 28: 7273-7283.
- Gargus JJ, Tournay A (2007) Novel mutation confirms seizure locus SCN1A is also familial hemiplegic migraine locus FHM3. *Pediatr Neurol* 37: 407-410.
- Frosk P, Mhanni AA, Rafay MF (2013) SCN1A mutation associated with intractable myoclonic epilepsy and migraine headache. *J Child Neurol* 28: 389-391.
- Yordanova I, Todorov T, Dimova P, Hristova D, Tincheva R, et al. (2011) One novel Dravet syndrome causing mutation and one recurrent MAE causing mutation in SCN1A gene. *Neurosci Lett* 494: 180-183.
- Escayg A, Heils A, MacDonald BT, Haug K, Sander T, et al. (2001) A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus--and prevalence of variants in patients with epilepsy. *Am J Hum Genet* 68: 866-873.
- Cestèle S, Labate A, Rusconi R, Tarantino P, Mumoli L, et al. (2013) Divergent effects of the T1174S SCN1A mutation associated with seizures and hemiplegic migraine. *Epilepsia* 54: 927-935.
- Marini C, Scheffer IE, Nabbout R, Suls A, De Jonghe P, et al. (2011) The genetics of Dravet syndrome. *Epilepsia* 52 Suppl 2: 24-29.
- Depienne C, Bouteiller D, Keren B, Cheuret E, Poirier K, et al. (2009) Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. *PLoS Genet* 5.
- Suls A, Jaehn JA, Kecskés A, Weber Y, Weckhuysen S, et al. (2013) De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. *Am J Hum Genet* 93: 967-975.
- Dibbens LM, Tarpey PS, Hynes K, Bayly MA, Scheffer IE, et al. (2008) X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment. *Nat Genet* 40: 776-781.
- Géczy J, Cloosterman D, Partington M (2006) ARX: a gene for all seasons. *Curr Opin Genet Dev* 16: 308-316.
- Wallace RH, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, et al. (2003) Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology* 61: 765-769.
- Polizzi A, Incorpora G, Pavone P, Ruggieri M, Annesi G, et al. (2012) Generalised epilepsy with febrile seizures plus (GEFS(+)): molecular analysis in a restricted area. *Childs Nerv Syst* 28: 141-145.
- Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, et al. (2007) The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain* 130: 843-852.
- Engel J (2001) International League Against Epilepsy (ILAE) A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 42: 796-803.
- Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, et al. (2010) Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 51: 676-685.
- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, et al. (2010) A method and server for predicting damaging missense mutations. *Nat Methods* 7: 248-249.
- Schwarz JM, Rödelberger C, Schuelke M, Seelow D (2010) MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* 7: 575-576.
- Guerrini R (2012) Dravet syndrome: the main issues. *Eur J Paediatr Neurol* 16 Suppl 1: S1-4.
- Escayg A, Goldin AL (2010) Sodium channel SCN1A and epilepsy: mutations and mechanisms. *Epilepsia* 51: 1650-1658.
- Marini C, Scheffer IE, Nabbout R, Mei D, Cox K, et al. (2009) SCN1A duplications and deletions detected in Dravet syndrome: implications for molecular diagnosis. *Epilepsia* 50: 1670-1678.
- Patino GA, Claes LR, Lopez-Santiago LF, Slat EA, Dondeti RS, et al. (2009) A functional null mutation of SCN1B in a patient with Dravet syndrome. *J Neurosci* 29: 10764-10778.
- Ogiwara I, Nakayama T, Yamagata T, Ohtani H, Mazaki E, et al. (2012) A homozygous mutation of voltage-gated sodium channel  $\beta$ (I) gene SCN1B in a patient with Dravet syndrome. *Epilepsia* 53: e200-203.
- Brunklaus A, Ellis R, Stewart H, Aylett S, Reavey E, et al. (2015) Homozygous mutations in the SCN1A gene associated with genetic epilepsy with febrile seizures plus and Dravet syndrome in 2 families. *Eur J Paediatr Neurol* 19: 484-488.
- Bolszak M, Anttonen AK, Komulainen T, Hinttala R, Pakanen S, et al. (2009) Digenic mutations in severe myoclonic epilepsy of infancy. *Epilepsy Res* 85: 300-304.
- Craig AK, de Menezes MS, Saneto RP (2012) Dravet syndrome: patients with co-morbid SCN1A gene mutations and mitochondrial electron transport chain defects. *Seizure* 21: 17-20.
- Striano P, de Jonghe P, Zara F (2013) Genetic epileptic encephalopathies: is all written into the DNA? *Epilepsia* 54 Suppl 8: 22-26.
- Bender AC, Morse RP, Scott RC, Holmes GL, Lenck-Santini PP, et al. (2012) SCN1A mutations in Dravet syndrome: impact of interneuron dysfunction on neural networks and cognitive outcome. *Epilepsy Behav* 23: 177-186.

31. McIntosh AM, McMahon J, Dibbens LM, Iona X, Mulley JC, et al. (2010) Effects of vaccination on onset and outcome of Dravet syndrome: a retrospective study. *Lancet Neurol* 9: 592-598.
32. Higurashi N, Nakamura M, Sugai M, Ohfu M, Sakauchi M, et al. (2013) PCDH19-related female-limited epilepsy: Further details regarding early clinical features and therapeutic efficacy. *Epilepsy Research* 106: 191-199.
33. Berkovic SF, Harkin L, McMahon JM, Pelekanos JT, Zuberi SM, et al. (2006) De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a retrospective study. *Lancet Neurol* 5: 488-492.
34. Saitoh M, Shinohara M, Hoshino H, Kubota M, Amemiya K, et al. (2012) Mutations of the SCN1A gene in acute encephalopathy. *Epilepsia* 53: 558-564.
35. Sakakibara T, Nakagawa E, Saito Y, Sakuma H, Komaki H, et al. (2009) Hemiconvulsion-hemiplegia syndrome in a patient with severe myoclonic epilepsy in infancy. *Epilepsia* 50: 2158-2162.
36. Takayanagi M, Haginoya K, Umehara N, Kitamura T, Numata Y, et al. (2010) Acute encephalopathy with a truncation mutation in the SCN1A gene: a case report. *Epilepsia* 51: 1886-1888.
37. Parisi P, Striano P, Trenité DG, Verrotti A, Martelletti P, et al. (2012) 'Ictal epileptic headache': Recent concepts for new classifications criteria. *Cephalalgia* 32: 723-724.
38. Kasperaviciute D, Catarino CB, Matarin M, Leu C, Novy J, et al. (2013) Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around SCN1A. *Brain* 136: 3140-3150.
39. International League Against Epilepsy Consortium on Complex Epilepsies (2014) Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies. *Lancet Neurol* 13: 893-903.